Histone deacetylase inhibitors containing chiral heterocyclic capping groups

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Declaration

I, Christopher James Matthews confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

C J Matthews

Abstract

Histone deacetylase (HDAC) enzymes are one of the best characterised epigenetic targets; aberrant activity of one or more of the 11 zinc-dependent HDAC isoforms has been associated with many different cancers, as well as several other diseases. Some HDAC inhibitors have been proven to be efficacious in treating cancer; three are FDA approved for treating cutaneous T-cell lymphoma or multiple myeloma. Animal models of other diseases have shown that HDAC inhibitors have potential as therapeutic agents. However the clinical use of HDAC inhibitors is currently limited by toxicity and side effects, amongst other problems, possibly arising because the inhibitors are not isoform selective. This thesis focuses on attempts to synthesise novel HDAC inhibitors with increased isoform selectivity.

A series of mocetinostat analogues substituted at the 4-poisition of the terminal 2-aminophenyl ring, was synthesised and tested for inhibition of HDAC isoforms, but no improvement over mocetinostat was found.

Subsequently multiple series of compounds containing a chiral heterocycle, in place of the pyrimidine ring in mocetinostat, were prepared and evaluated against a selection of HDAC isoforms. The development of highly convergent methodology enabled the rapid synthesis of these chiral mocetinostat analogues. Several compounds were discovered that are more potent and selective inhibitors than mocetinostat against HDAC3. Those compounds containing a 2-amino-linked dihydrooxazole ring were the most potent, and in all cases the amino-linked heterocycle outperformed its thioether-linked analogue. Mocetinostat and a novel chiral analogue were shown to be slow, tight-binding inhibitors of HDAC2 and HDAC3.

Attempts were made to discover a novel zinc-binding group; a selection of potential bi-dentate zinc-chelating fragments was assayed and 8-hydroxyquinoline emerged as a promising candidate for HDAC8 inhibition. Accordingly some substituted 8-hydroxyquinolines were prepared in order to conduct a brief SAR study of the novel zinc-binding group, however no improvement on the unsubstituted 8-hydroxyquinoline was found.

Acknowledgements

I have many people to thank, without these people this thesis would not have been possible, there are too many people to name but I will try to name some and I apologise for any that I miss.

Firstly I'm incredibly indebted to my Mum and Dad, for always encouraging me to set my sights high and achieve as much as I could; thank you for supporting me through everything in my life. Not only my parents but I would like to thank my brothers, for being good examples (mostly) and more recently my sisters in law and my nieces and nephews too for understanding that I've *been busy*, you guys are all the best.

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Lastly I am most grateful to Reggie for going fishing.

Contents

Abbreviations

δ	chemical shift
Ac	acetyl
ADP	adenosine diphosphate
AMC	7-amin-4-methylcoumarin
anh.	anhydrous
aq.	aqueous
Ar	aryl
AUC	area under the curve
Bn	benzyl
Boc	*tert*-butoxycarbonyl
BOP	benzotriazole-1-yl-oxy-tris-(dimethylamino)-phosphonium hexafluorophosphate
br.	broad (spectral)
Bu	butyl
CI	chemical ionisation
d	doublet (spectral)
DCM	dichloromethane
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
DIBAL	diisobutylaluminium hydride
DIPEA	diisopropylethylamine
DMF	dimethylformamide
DNA	deoxyribonucleic acid
EDC	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
eq.	equivalent(s)
EI	electron impact ionization
ES	electrospray ionisation
Et	ethyl
FDA	US food and drug administration
HRMS	high resolution mass spectrometry
h	hour(s)
HAT	histone acetyl transferase
HDAC	histone deacetylase enzyme
HMT	histone methyltransferase
HOBt	hydroxybenzotriazole

HPLC	high performance liquid chromatography
IC_{50}	half maximal inhibitory concentration
IR	infrared
J	coupling constant (Hz)
m	multiplet (spectral)
MDAP	mass directed auto purification
Me	methyl
min	minute(s)
mp	melting point
m/z	mass:charge ratio
n	normal
NAD^+/H	nicotinamide adenine dinucleotide oxidised/reduced
NBS	*N*-bromosuccinimide
NCoR	nuclear receptor co-repressor
NMR	nuclear magnetic resonance
PDB	protein data bank
Ph	phenyl
ppm	parts per million
rt	room temperature
s	singlet (spectral)
SAR	structure-activity relationship
SUMO	small ubiquitin-like modifier
t	triplet (spectral)
t	(*tert*) tertiary
TBS	*tert*-butyl dimethylsilyl
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin-layer chromatography
TSA	trichostatin A
ν_{max}	IR absorption maxima

1 Introduction

1.1 Project Aims

This thesis focuses on attempts to synthesise an isoform selective HDAC inhibitor, the enzyme targeted specifically is HDAC11 (the most recently discovered, and smallest member of the HDAC family). Other than targeting HDAC11, the aim is to synthesise a HDAC inhibitor with improved isoform selectivity for one isoform, compared to the current best inhibitors.

The purpose of developing an isoform selective HDAC inhibitor is twofold, firstly to use it as a 'tool compound' that can be used to probe the biological processes associated with that specific isoform, and to find out if that specific isoform is responsible for, or at least directly implicated in disease development or progression. The second reason for preparing an isoform selective HDAC inhibitor is so that it can be utilised as a therapeutic agent, although the aim of this project is only the discovery of a lead compound, not to develop a clinical therapeutic agent. The desire for an isoform selective HDAC inhibitor as a therapeutic agent is driven by the desire for a better safety profile compared to current pan-selective HDAC inhibitors.

The methods used to achieve isoform selectivity will focus on three different concepts; modification of known zinc-binding groups (particularly extension of the benzamide zinc-binding group into the acetate-release pocket), discovery of a new zinc-binding group (with inherent isoform selectivity) and introduction of a chiral centre into the cap region (to exploit subtle differences in the three-dimensional surfaces of the different isoforms).

The compounds prepared will undergo different evaluations, including measuring basic physiochemical properties to check their suitability for the desired final purposes (e.g. *in vivo*). Compounds will also be tested against a panel of different recombinant HDAC isoforms in an *in vitro* assay, in order to identify those compounds which have some isoform selectivity. A selection of the more promising compounds will also be tested in a cell based assay, to investigate HDAC inhibition in a cellular environment.

1.2 HDACs and their cellular role

Histone deacetylase enzymes (HDACs) are a class of enzymes that catalyse the deacetylation of lysine residues in the amino terminal tails of core histones. Histones are basic proteins that in their protonated forms bind to the negatively charged phosphate backbone of the DNA double helix, forming a complex with DNA known as chromatin.[1] The DNA within this chromatin structure is highly compact, and it is not possible for the transcriptional machinery to access the genetic information when the chromatin is in this condensed form.[2] There are several methods by which genetic information can be made available for transcription, including post-translational modification of the histone proteins. Histones contain a high proportion of lysine and arginine residues which makes these proteins particularly susceptible to a number of post-translational modifications.[3] Post-translational modifications include acetylation of lysine residues by histone acetyltransferases (HATs), sumoylation of lysine residues by the small ubiquitin-like modifier (SUMO), methylation of lysine and arginine residues by histone methyltransferases (HMTs), serine phosphorylation by serine kinases, ubiquitination by ubiquitin ligases, and poly ADP-ribosylation by poly ADP-ribose polymerase.[4] All known covalent histone modifications are reversible, and therefore the extent to which a modification is present relies on a dynamic equilibrium, with enzymes responsible for both attachment and removal of the modifying group present in the nucleus at the same time.[5]

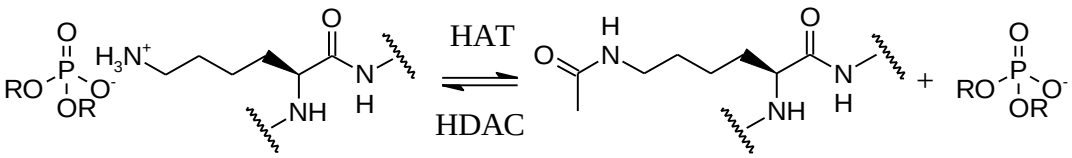


Figure 1.2-1: *Equilibrium of competing HAT vs HDAC*

Of these modifications, acetylation is one of the best characterised; several classes of proteins have been identified as histone acetyltransferases (HATs) each displaying different specificities.[1] When a lysine residue becomes acetylated, it is no longer protonated and therefore it cannot bind to the phosphate backbone of the DNA which in turn gives rise to a less condensed region of chromatin that is more accessible to the transcriptional machinery.[6] The competing deacetylation reaction is catalysed by HDACs and it is the balance of these two processes (**Figure 1.2-1**), acetylation and deacetylation that control the openness of the chromatin structure and therefore the transcriptional status of the genetic information. Hypoacetylated (under-acetylated) chromatin is very condensed and leads to gene silencing,[7] whereas hyperacetylaed

(over-acetylated) chromatin is very open and leads to increased gene expression.[8] Acetylation also affects the recruitment of DNA binding factors, for example the bromodomain 'recognises' acetylated lysines and binds to them. This bromodomain is present in over 40 human proteins and these proteins are involved in recruiting other transcription factors. Bromodomains have received considerable attention recently from the medicinal chemistry community as another epigenetic target for treating diseases.

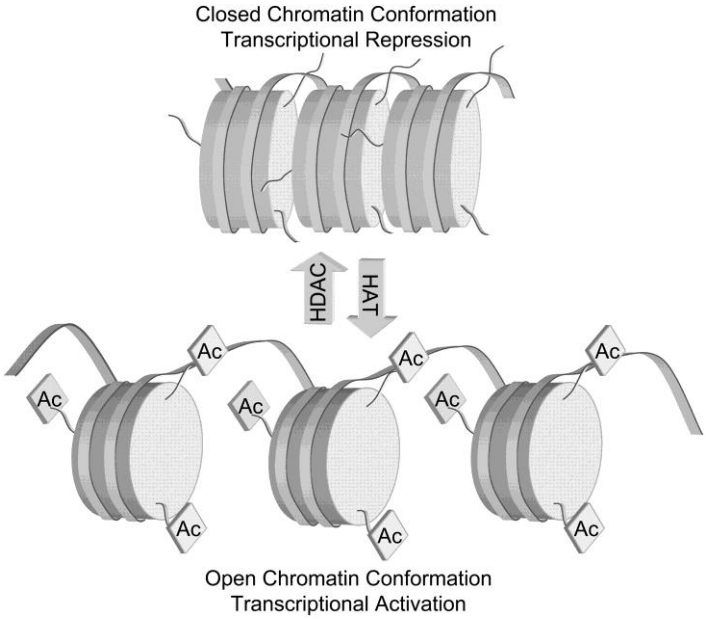


***Figure 1.2-2**: Modification of chromatin structure and transcriptional status by HDAC and HAT.*[9]

There are 18 different enzymes which have been found to deacetylate acetylated histones in mammals and they are grouped into classes based on their primary sequences. Eleven of these enzymes share a highly conserved zinc dependent active site, and it is these enzymes which are known as HDACs 1-11. The other 7 enzymes have an NADH dependence and are known as the sirtuin proteins (or class III HDACs), this project does not concern these enzymes since their different mechanism allows them to be unaffected by the majority of HDAC inhibitors. The 11 HDACs are further split into different classes based on their homology to two yeast proteins: class I (1, 2, 3 and 8) are most closely related to *S. cerivisiae* transcriptional regulator Rpd3, class II (4, 5, 6, 7, 9 and 10) are more similar to yeast deacetylase Hda1. HDAC11 is the most recently identified HDAC enzyme and it is the only member of class IV; it is not very homologous to either Rpd3 or Hda1 and so it does not fit well into class I or II.

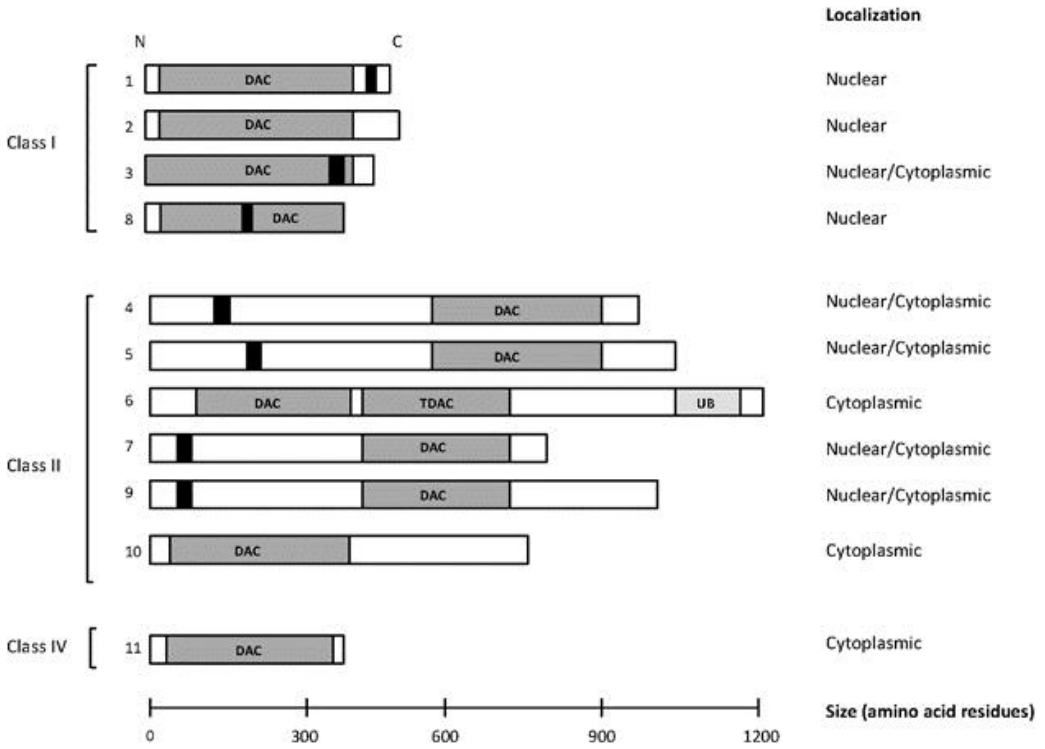


Figure 1.2-3*: Comparison of HDAC isoforms. Length of bars indicate the length of protein, the light grey is the location of the deacetylase domain (DAC) and black is the nuclear localisation domain. The sub-cellular localisation is indicated on the right. TDAC; tubulin deacetylase domain, UB; ubiquitin binding.*[10]

In vitro, HDACs predominantly function in multi-protein complexes which include DNA-binding, chromatin remodelling and recruitment proteins. Recombinant HDACs that are expressed in human cells or other eukaryote cell expression systems, such as insect cells, are functionally active. Although in some cases co-expression of the HDAC with the deacetylase activation domain of the complex as a fusion protein gives the HDAC an increase in activity approximately 100 fold. Typically HDACs expressed in prokaryotes are not functionally active, with the exception of HDAC8 which lacks the C-terminal tail associated with (functionally activating) post-translational modifications in other HDACs.

The first crystal structures published were of HDAC8 in 2004 by two separate groups, all of them with the protein bound to a hydroxamic acid inhibitor.[11,12] Since then many more crystal structures of HDAC8 have been published and recently crystal structures of other HDAC isoforms have also been published. Currently there are crystal structures in the protein data bank for HDAC1, 2, 3, 4, 7, and 8 (of the deacetylase domain at least). This opens up the possibility of using computational techniques and *in silico* docking to aid the development and design of HDAC inhibitors.

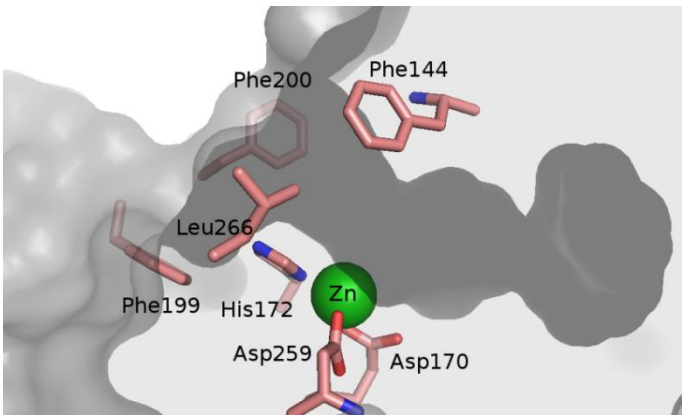


***Figure 1.2-4**: Active site of HDAC3 (PDB 4A69) with residues bound to zinc (green) and those lining the tunnel shown in pink. The surface of the tunnel is shown in dark grey and the acetate-release pocket can be seen extending to the right of the zinc.*

The active site of all HDACs is very highly conserved; it consists of a zinc atom that is buried between 7 and 8 Å within a narrow (~5 Å diameter) hydrophobic tunnel. The zinc atom is bound by the carboxylates of two conserved aspartic acid residues and by a conserved histidine (**Figure 1.2-4**). It is thought that the carbonyl oxygen of the acetyl lysine and a water molecule also coordinate to the zinc ion during the catalytic cycle (**Figure 1.2-5**). This makes the carbonyl more electrophilic and the water molecule is also made more nucleophilic by the neighbouring aspartate-histidine charge relay system. The zinc ion also stabilises the tetrahedral intermediate, which HDAC inhibitors aim to mimic with their zinc-binding groups.

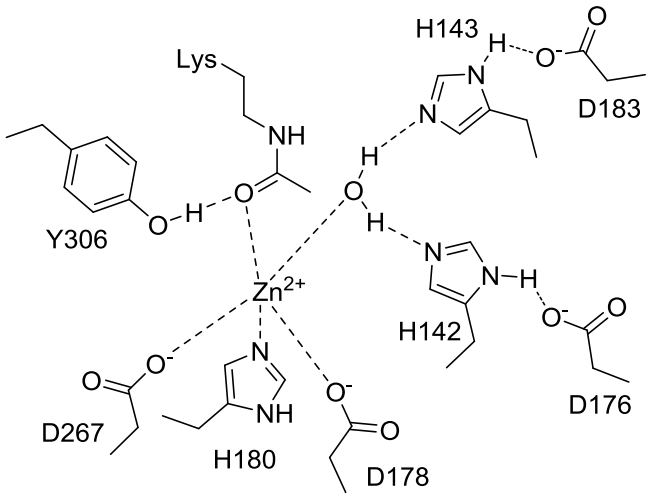


***Figure 1.2-5**:Schematic diagram showing the proposed model of how the acetylated lysine would interact with the HDAC8 catalytic machinery.*[11]

1.3 HDACs and disease

Aberrant expression and activity of HDACs has been associated with many different diseases and even many different disease types. In many cases, animal models

of these diseases have shown that after removing the corresponding HDAC activity, by either gene knock down of the HDAC isoform or by expression of an inactive variant of the isoform, the symptoms of the disease can be alleviated. This suggests that inhibitors of HDAC enzymes have the potential to treat these diseases,[13,14] and HDAC inhibitors have been investigated as therapeutic agents for treating a range of disorders. However because of toxicity and the side effects associated with many HDAC inhibitors (possibly due to their lack of isoform selectivity), cancer is the disease area that has been most investigated. A brief discussion about the role of HDACs in cancer, and then in a selection of other diseases will follow.

1.3.1 Cancer

A disease that results from unregulated cellular proliferation, and has the potential to spread to other parts of the body, is known under the broad term cancer. Tumours may form when cells stop being correctly controlled by the mechanisms that regulate normal cell growth. However tumours can be benign or malignant, the difference being that benign tumours are not classified as cancerous because they lack the ability to metastasise (invade adjacent or distant tissues), since they have retained control of differentiation.

HDACs play an important role in cell-cycle progression and differentiation, since loss of acetyl groups from lysine residues cause decreased gene expression and reduced DNA repair.[15,16] Overexpression of HDACs and the resulting histone hypoacetylation is associated with several cancers in a range of different human tissues.[17] Additionally, HDACs can drive formation and growth of cancers by being abnormally recruited to certain gene promoting complexes by oncogenic fusion proteins.[18]

Some HDAC inhibitors transform cancer cells into non-proliferating cells (normal phenotype) by inducing differentiation. It is also known that HDAC inhibitors cause growth inhibition and induce apoptosis (programmed cell death), as well as reducing angiogenesis (growth of blood vessels).[19] Growth inhibition and apoptosis, induced by HDAC inhibitors, are often linked to up-regulation of p21, a tumour suppressor protein.[20]

Individual class I HDAC isoforms have been implicated in various different cancers; HDAC1 and HDAC2 were widely believed to be the primary targets of HDAC inhibitors in many cancers. For example it has been shown that HDAC 1, 2 and 3 are highly expressed in prostate cancers.[21] Other isoforms have also been linked to some

cancers; HDAC3 has been shown to be aberrantly recruited to the promoters of oncogenes in acute myeloid leukaemia.[22] HDAC8 has been identified as transcriptional regulator of p53 expression, mutation of the *p53* gene is the most common genetic alteration in human cancer and it contributes to making cells resistant to anticancer therapy.[23]

HDAC11, the most recently discovered HDAC, has also been identified as a novel drug target in many cancers, it is overexpressed in colon, prostate, breast, and ovarian cancer cell lines. Furthermore HDAC11 depletion in these cancer cell lines caused cell death and reduced metabolic activity, whereas in normal cells HDAC11 depletion caused no changes in metabolic activity and viability.[24] In Hodgkin's lymphoma, a cancer originating from white blood cells (lymphocytes), HDAC11 is essential for regulating OX40L expression (OX40L is a member of the tumour necrosis factor family of proteins, and it is required to induce an antitumour immune response). Knockdown of HDAC11 in three Hodgkin's lymphoma cell lines resulted in up-regulated OX40L expression and induction of apoptosis.[25]

1.3.2 Other diseases

Inflammatory diseases share some characteristics with cancer, since the tumour microenvironment is dominated by inflammation, and both involve the response of the immune system.[26] Inflammatory disorders cover a wide range of diseases, including asthma, rheumatoid arthritis and autoimmune diseases such as inflammatory bowel disease.

HDAC11 has been demonstrated to be a negative transcriptional regulator of the gene encoding for interleukin 10 expression in mouse and human antigen presenting cells. Interleukin 10 is an anti-inflammatory signalling protein; it has a key role in inducing tolerance and preventing self-tissue damage. Since HDAC11 depletion gives rise to increased levels of IL-10, selective inhibition of HDAC11 could provide a novel therapeutic target for chronic inflammatory diseases.[27]

A recent review on the use of HDAC inhibitors in the treatment of asthma concludes by stating that the results of several *in vivo* and *ex vivo* allergic models show HDAC inhibitors to have therapeutic promise. However the mechanisms of action are not fully understood since both pro- and anti-inflammatory effects are observed in different cell types, and treatment with HDAC2 activators can improve corticosteroid response.[28] In the context of developing asthma therapeutics it is important to consider the effects of HDAC inhibitors with increased isoform selectivity.[29]

An analysis of peripheral blood mononuclear cells from rheumatoid arthritis patients showed significantly increased HDAC activity compared to cells from healthy individuals. Treatment with a HDAC inhibitor caused reduced production of tumour necrosis factor and interlukin-6 (a pro-inflammatory cytokine) in these cells.[30]

Multiple studies on mouse models of colitis have shown that HDAC inhibitors can offer protection, as assessed by a variety of measures including macroscopic changes, such as increased colon lengths and weight gain, and also changes in levels of inflammatory cytokines.

There is a wealth of growing evidence that links neurodegenerative conditions *in vivo* and *in vitro* with histone hypoacetylation. In many disease models treatment with HDAC inhibitors protect against neurodegeneration and normalise the levels of histone acetylation. Models of the following diseases have shown promising results by treatment with HDAC inhibitors: motor neurone disease, Huntingdon's disease, stroke, Parkinson's disease, and Alzheimer's disease.[31]

Recently HDAC2 has been implicated in memory updating, and neuroplasticity, HDAC2 inhibition epigenetically primes the expression of neuroplasticity-related genes. If applied during memory reconsolidation this enables the attenuation of remote fear memories, and could provide a therapeutic option for those suffering from post-traumatic stress disorder.[32]

Friedreich's ataxia is a progressive neurodegenerative disease caused by a defect in transcription that results in deficiency of frataxin (an essential mitochondrial protein) resulting in symptoms that include lack of coordination, slurred speech and decreased sensory function. Certain HDAC inhibitors (benzamides) reverse the gene silencing associated with Friedreich's ataxia and increase frataxin levels, but hydroxamic acid HDAC inhibitors vorinostat and TSA have no effect on the transcription of the frataxin gene.[33] Subsequently HDAC3 was identified as the target isoform, and the variation between inhibitor types was attributed to the differences in kinetics of binding (see section 1.4.2, p 29).[34,35]

1.4 HDAC inhibitors

Natural product Trichostatin A (TSA) (**Figure 1.4-3**) was the first HDAC inhibitor, identified 20 years ago (1995),[36] since then there has been much progress towards understanding HDAC enzymes and many HDAC inhibitors have been discovered. Inhibitors of HDACs usually fit the well-defined pharmacophore that

consists of a zinc-binding group joined by a non-polar linker group to a cap group (or surface recognition group) illustrated with vorinostat and mocetinostat in **Figure 1.4-1**.

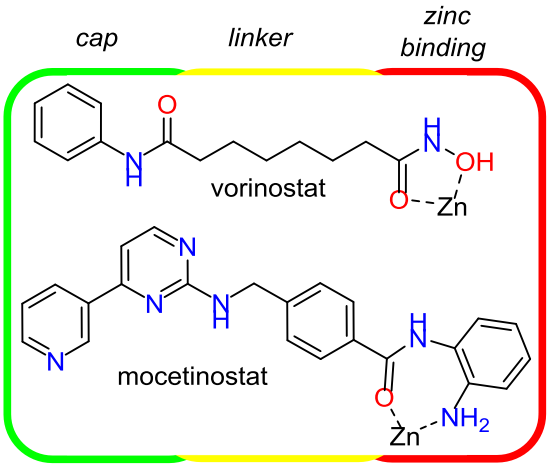


***Figure 1.4-1**: Pharmacophore for HDAC inhibitors illustrated with vorinostat and mocetinostat.*[37]

One class of inhibitors that does not appear to fit the pharmacophore is cyclic peptides such as romidepsin (Istodax, FK228), a natural product isolated from *Chromobacterium violaceum* (Figure 1.4-2) used in the treatment of peripheral T-cell lymphoma.[38] Romidepsin does however fit the model described here; it contains a latent zinc-binding group. The disulfide bridge is reduced in cells and the longer-chain thiol that is released becomes the zinc-binding group, making romidepsin a natural prodrug. The cyclic peptide moiety is a large surface recognition group which contains hydrophobic amino acid residues and the same is true of other cyclic peptide HDAC inhibitors.[39]

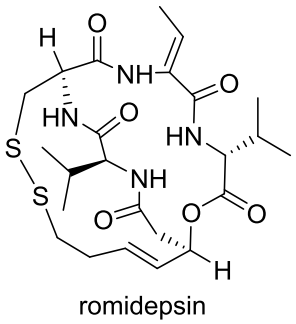


Figure 1.4-2: Romidepsin a bicyclic depsipeptide HDAC inhibitor.

There are some inhibitors that do not fit the pharmacophore and the most common deviation is inhibitors that extend beyond the zinc-binding group. In many of the HDAC crystal structures the active site tunnel continues into the protein past the zinc atom. This cavity is sometimes referred to as the 'back pocket' or as the 'acetate-release pocket'. It has been postulated that this internal cavity is involved in shuttling an

acetate molecule, the side product formed from deacetylation, away from the active site and conversely it has been postulated to play the opposite role of bringing the water molecule into the active site.[40] Since the different isoforms are less well conserved in this region, it has been suggested that isoform selective inhibition could be achieved by targeting the acetate-release pocket.[41] There have been some HDAC inhibitors made that extend into the acetate-release pocket (**Figure 1.2-4**) such as 5-substituted *N*-2-aminophenyl benzamides discussed in more detail in section 1.4.2 (**Table 1.4-6** and **Table 1.4-7**).

Small molecule HDAC inhibitors are often grouped into classes characterised primarily by the type of zinc-binding group such as hydroxamic acids, carboxylates, and benzamides (**Figure 1.4-3**). Inhibitors are also grouped into subclasses of these groups by other structural features such as a cyclic or non-cyclic linker region.

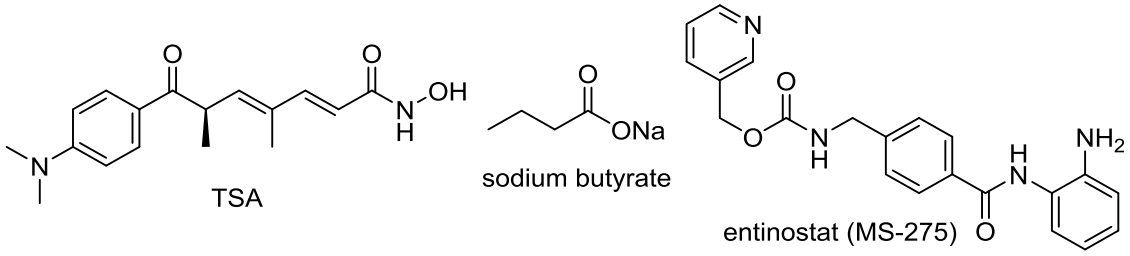


***Figure 1.4-3**: Some well-known HDAC inhibitors showing different zinc-binding groups.*

The hydroxamic acid binding group is the most widely explored zinc-binding group, and the majority of HDAC inhibitors that have been in clinical trials are hydroxamic acid based inhibitors.[42–44] Indeed the first HDAC inhibitor approved by the FDA was a hydroxamic acid; vorinostat (Zolinza^{TM}, SAHA), which has been approved for once-daily oral treatment of advanced cutaneous T-cell lymphoma, a rare cancer of the immune system.[45] More recently two more hydroxamic acid HDAC inhibitors, belinostat (Belodaq^{TM}) and panobinostat (Farydak^{TM}) have been approved for peripheral T-cell lymphoma and multiple myeloma respectively.[46,47] The hydroxamic acid binding group binds with a high affinity to zinc giving rise to inhibitors with high potency. However hydroxamic acids have poor pharmacokinetic properties and some toxic effects *in vitro* because the hydroxamic acid group binds other metal ion-containing proteins such as matrix metalloproteinases. In spite of this there has been a great deal of structure-activity relationship (SAR) studies of hydroxamic acid compounds focusing on different linker groups and different cap groups. Optimisation of the linker length in both straight chain and cyclic linker compounds has been carried out, together with studies into the size and nature of the cap group. Although there has been such a large

scale investigation into hydroxamic acid inhibitors the problems of toxicity and selectivity are not easily overcome. These problems can be tolerated when developing a treatment for a rare cancer because side effects are not such a large concern when developing a drug that is aiming to extend a patient's life by several months. For the treatment of other diseases, especially chronic diseases such as asthma, arthritis, or inflammatory bowel disease, side effects pose a far more substantial problem. Therefore, if a HDAC inhibitor is to be developed for the treatment of such diseases a hydroxamic acid seems unlikely to provide the necessary selectivity and safety profile.

1.4.1 Zinc binding groups

Other simple zinc-binding groups bind the zinc ion less tightly and as a result typically give lower potency inhibitors, for example a carboxylate group, found in valproic acid (VPA) and sodium butyrate (**Figure 1.4-3**), and thiols such as the thiol analogue of vorinostat.[48] Another popular zinc-binding group is the *N*-(2-aminophenyl)benzamide group found in entinostat (**Figure 1.4-3**) discussed in more detail in the following section (1.4.2). There have been several other potent zinc-binding groups discovered such as trifluoromethyl ketones,[49] *alpha*-keto amides,[50] heterocyclic ketones,[51] and some more recently such as; (*R*)-α-amino amide as in **1**,[52] the α-oxime amide **2**,[53] and the hydroxypyrimidine **3**.[54]

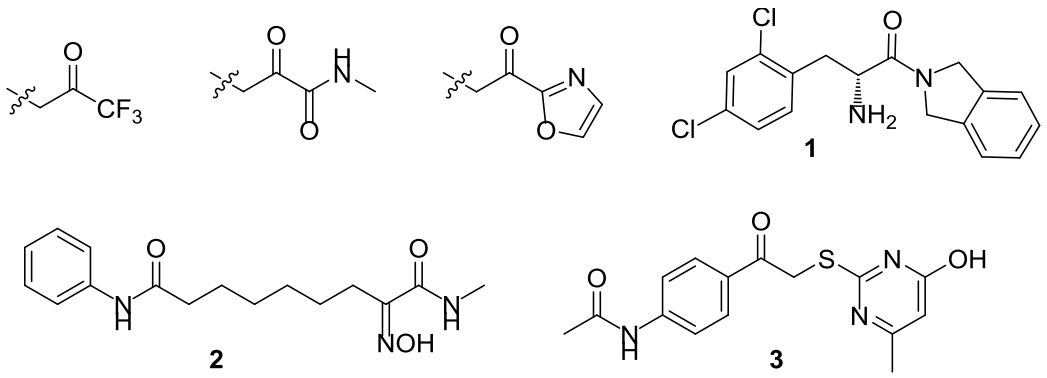


***Figure 1.4-4**: Other examples of zinc-binding groups.*

Computational studies have been carried out in order to try and identify novel zinc-binding groups that would give potent HDAC inhibitors. One recent study by Chen *et al.* initially focussed on the calculated binding energies of a series of potential chelating fragments to zinc in a small active site model.[55] Promising fragments were then docked into a full active site model (HDAC2 - PDB 3MAX) to check for steric clashes. One interesting fragment was 8-hydroxyquinoline, which in the initial model had a binding energy similar to that of a hydroxamic acid (-23.5 *cf.* -23.7 kcal/mol) but

had steric clashes in the full model with HDAC2, so it was not explored further.

A search of the literature for previous reports of 8-hydroxyquinoline HDAC inhibitors gave one result which showed the potential of this unit as a HDAC inhibitor; treatment of HeLa cell nuclear extract (which contains a variety of HDAC enzymes) with 500 μM 8-hydroxyquinoline gave 15% inhibition (comparable to sodium butyrate).[56]

Bora-Tatar *et al.* were primarily interested in carboxylic acids as HDAC inhibitors, however many of their carboxylic acids tested also contained a catechol unit, or in some cases an *ortho*-methoxy phenol. The authors suggest that the HDAC inhibitory activity is due to carboxylate binding the zinc atom, however it seems likely that in some cases at least the catechol moiety is responsible for binding to zinc since in the computational study by Chen *et al.* both catechol and *ortho*-methoxy phenol had shown promising zinc-binding energies (-19.6 and -24.2 kcal/mol respectively).[55] **Table 1.4-1** shows some of the relevant compounds in mention and the percentage inhibition (measured by Bora-Tatar *et al.*) of HeLa nuclear extracts.

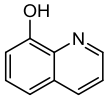
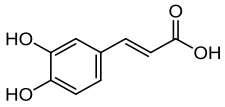
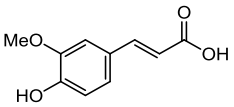
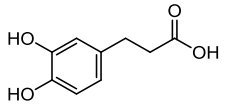
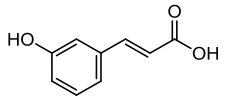
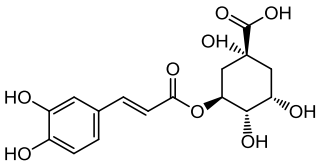
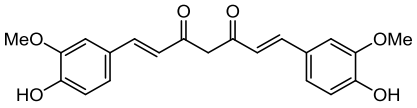
Name	Structure	Inhibition at 500 μM (%)
8-hydroxyquinoline		15
caffeic acid		20
ferulic acid		20
dihydrocaffeic acid		14
3-hydroxycinnamic acid		4
chlorogenic acid		60 *(375 μM)
curcumin		48 *(115 μM)

***Table 1.4-1**: Inhibition of HeLa nuclear extracts.*
** IC_{50} values against HeLa nuclear extracts.*[56]

This indicated that catechol might also be an interesting zinc-binding group,

however catechol is known to be a promiscuous metal binding group and therefore catechol containing structures are generally avoided by medicinal chemists. An analysis of this data did suggest that 8-methoxy quinolines could be an effective zinc-binding group since the *ortho*-methoxy phenol ferulic acid was as potent as caffeic acid, and curcumin (also an *ortho*-methoxy phenol) was the most potent compound tested (based on its IC_{50}). However both of these compounds still contain a free, non-methylated phenol which could be deprotonated in the active site and may make a significant contribution to the binding of zinc which would not be possible with an 8-methoxy quinoline.

1.4.2 Benzamide inhibitors

As mentioned above the *N*-(2-aminophenyl)benzamide zinc-binding group has become a popular choice for HDAC inhibitors and these inhibitors are often known as benzamide inhibitors. The name benzamide does not well describe the zinc-binding group of these inhibitors and in fact the benzamide unit (i.e. an amide derived from a benzoic acid) is not actually required; it is the *N*-(2-aminophenyl)amide (*ortho*-aminoanilide) unit which is essential. *N*-(2-aminophenyl)amides derived from vinyl, propargyl, and even alkyl carboxylic acids have also been shown to be HDAC inhibitiors.[57,58] In spite of this, these *N*-(2-aminophenyl)benzamide inhibitors will be referred to as benzamide inhibitors throughout this thesis in the interest of brevity and to follow what has become convention.

It is now well known that benzamide HDAC inhibitors usually selectively inhibit class I enzymes more than class II enzymes and this has made benzamide compounds attractive to those seeking to develop HDAC1/2 inhibitors as cancer therapeutics. As a result of this, a huge number of different benzamide compounds have been prepared in recent years, and often a large series of very close analogues was made by a company with only sparse amount of enzyme inhibition data published. A selection of these benzamide inhibitors will now be discussed.

Shown in **Table 1.4-2** is a recent report of the full isoform selectivity profile of the first benzamide inhibitor reported (entinostat) and a close analogue chidamide (entries 1 and 2). This clearly highlights the class I (HDAC1, 2, 3, and 8) selectivity of benzamide inhibitors and in particular the higher potency against HDAC1, 2 and 3. Chidamide (Epidaza^{TM}) has recently been approved in China for the treatment of peripheral cutaneous T-cell lymphoma, which represents a breakthrough since it is the first benzamide HDAC inhibitor approved for use in the clinic and the first orally

bioavailable HDAC inhibitor.[59] Entries 3 and 4 of **Table 1.4-2** highlight the selectivity that can be achieved using a benzamide zinc-binding group compared with a hydroxamic acid. In this case the linker is aliphatic, and the bulky cap group confers even greater selectivity for HDAC3. It is relatively uncommon to find inhibition data against all eleven isoforms which is (in part) because assays against all isoforms were not readily available in the past, and even now that they are available, testing against all isoforms is expensive.

Entry	Compound	HDAC IC_{50} (μM)										
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
1	MS-275	0.26	0.31	0.50	>30	>30	>30	>30	2.7	>30	0.25	0.65
2	HBI-8000	0.095	0.16	0.067	>30	>30	>30	>30	0.73	>30	0.078	0.43
3	ST2741	0.73	2.0	0.35	1.3	0.59	0.006	0.53	0.24	0.64	0.87	0.43
4	ST3071	21.0	1.1	0.260	150.0	28.0	*	*	74.0	50.0	73.0	5.80

***Table 1.4-2**: Isoform selectivity profiles of some benzamide inhibitors and comparison with a hydroxamic acid analogue, * not determined.*[58,60]

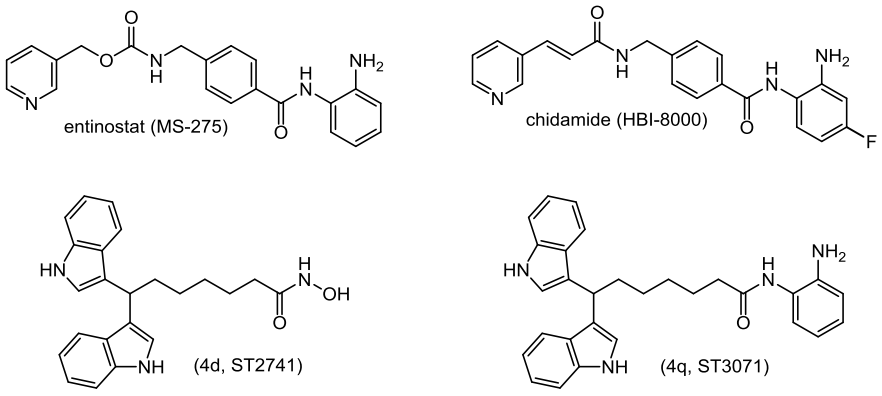


***Figure 1.4-5**: Structures of compounds in **Table 1.4-2**.*

The following three compounds (**Table 1.4-3**) show that very small chemical changes can have a significant effect *in vitro;* they are taken from a patent by Orchid Research Laboratories Limited which contained a large number of benzamide HDAC inhibitors.[61] Modifying a substituent on a phenyl ring in the cap group gave interesting results, methoxy to fluoro has a 1.2 fold decrease in IC_{50}, but methoxy to chloro has a 4 fold decrease in IC_{50}. However, only HDAC1 IC_{50} values were reported and so any effect on selectivity is unknown.

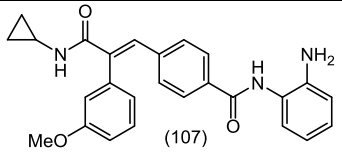
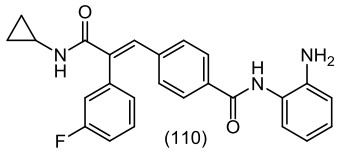
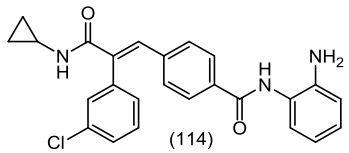
Entry	Compound	HDAC1 IC_{50} (μM)
1	 (107)	0.180
2	 (110)	0.150
3	 (114)	0.044

***Table 1.4-3**: Inhibition of HDAC1 comparing small changes to the cap group.*[61]

In addition to some *in vitro* enzyme assay data, some authors also report some cancer cell growth inhibition data such as MTT HCT116 (**Table 1.4-4** and **Table 1.4-5**) which is an antiproliferative assay using a human colon cancer cell line. The compounds in **Table 1.4-4** show that heterocycles can occupy the cap region, however the correct length of inhibitor is crucial. Compounds in which the heterocycle is appended with another phenyl ring have a roughly 10 fold decrease in IC_{50}, both in the *in vitro* HDAC1 assay, and the cell line assay (**Table 1.4-4**, entries 1 and 2 *cf.* entries 3 and 4).

Whilst the compounds in **Table 1.4-4** showed a good correlation between the *in vitro* enzyme assay data and the cancer cell line data, this is not always the case as illustrated in **Table 1.4-5**. In these examples some modifications which increase potency against purified recombinant HDAC1 actually decrease the antiproliferative effect in the cell line assay (entry 3 *cf.* entry 4). These inhibitors have the same straight chain alkyl linker section and different cap groups, some aromatic and some heteroaromatic. It is also worth noting the difference in the HDAC1 IC_{50} of entinostat reported here (1 μM) compared with **Table 1.4-2** entry 1 (0.26 μM) and **Table 1.4-7** entry 3 (0.54 μM). This highlights that in general, comparing IC_{50} values from different publications is very difficult because there is a large degree of variability in the numbers reported (even for the same compound and isoform).

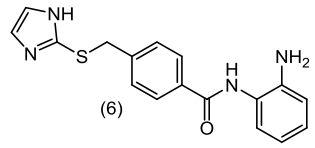
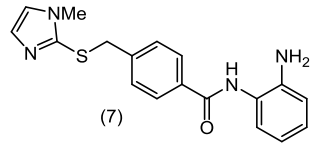
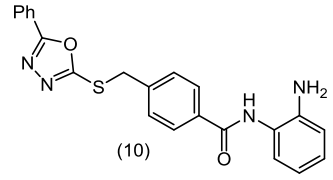
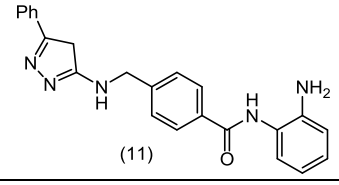
Entry	Compound	HDAC1 IC_{50} (μM)	MTT HCT116 IC_{50} (μM)
1	 (6)	0.5	5
2	 (7)	1	5
3	 (10)	0.08	0.4
4	 (11)	0.07	0.3

***Table 1.4-4**: Inhibition of HDAC1 and HCT116 cells by benzamide inhibitors with heterocyclic cap groups.*[62]

Following this survey of different cap groups commonly found in benzamide HDAC inhibitors, inhibitors probing the acetate-release pocket (discussed earlier and shown in **Figure 1.2-4**) will be discussed. In the first report of entinostat (MS-275), a methyl substituent was 'walked' around the terminal amino-phenyl ring to test whether substitution could be tolerated on that ring. It was found that only the 5-methyl substituted 2-aminophenyl ring maintained inhibition of HDAC activity, and was slightly more potent (tested against a mixture of HDACs obtained from the cell lysate of human leukaemia cells). It was also found that 2-hydroxyphenyl is slightly more potent than 2-aminophenyl (**Table 1.4-6**: entry 5 *cf.* entry 1), and this has since been shown to be the case generally.

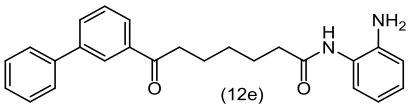
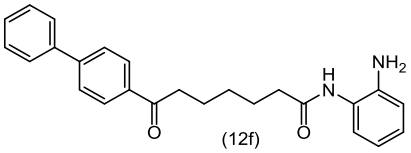
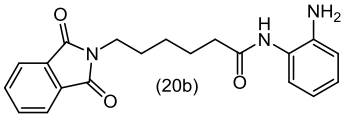
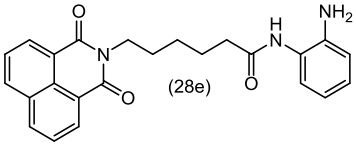
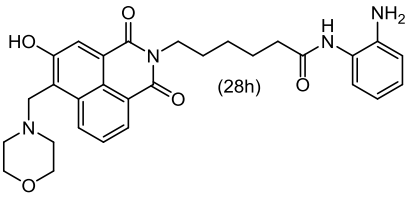
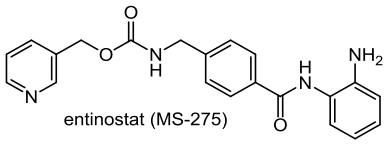
Entry	Compound	HDAC1 IC_{50} (μM)	MTT HCT116 IC50 (μM)
1	 (12e)	1	12
2	 (12f)	2	7
3	 (20b)	2	24
4	 (28e)	3	2
5	 (28h)	0.5	1
6	 entinostat (MS-275)	1	0.5

Table 1.4-5*: Inhibition of HDAC1 and HCT116 cells by benzamide inhibitors with aromatic or heteroaromatic cap groups.*[63]

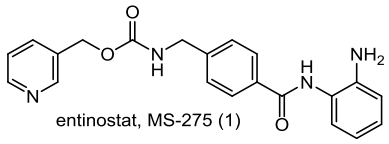
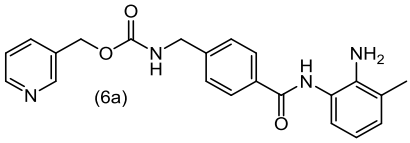
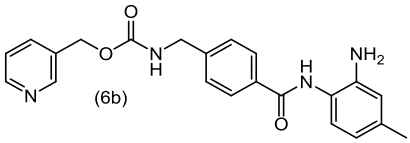
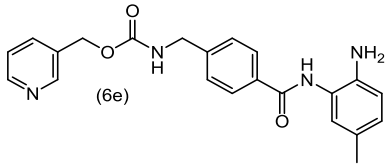
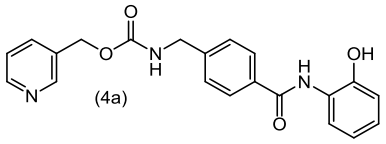
Entry	Compound	Mixture of HDACs IC_{50} (μM)
1	 entinostat, MS-275 (1)	4.8
2	 (6a)	>100
3	 (6b)	>100
4	 (6e)	2.8
5	 (4a)	2.2

Table 1.4-6*: Inhibition of HDAC activity from human leukaemia K562 cells.*[64]

Subsequently other research groups have explored 5-substitution of the terminal 2-aminophenyl ring. The data shown below (**Table 1.4-7**) is taken from some work carried out by MethylGene, when trying to exploit sulfur-sulfur interactions with methionine and cysteine residues lining the walls of the acetate-release pocket. Some thienyl substituted benzamide inhibitors were prepared and an increase in potency against HDAC1 and HDAC2 was observed. Furthermore inhibition of HDAC3 and HDAC8 was reduced in the thienyl substituted compounds, such as CI-994 (**Table 1.4-7**, entry 1 *cf.* entry 2). Therefore introduction of a thienyl group at the 5-position of the 2-aminophenyl ring increases isoform selectivity towards HDAC1 and 2. The effect of thienyl substitution was maintained even with different cap groups such as the carbamate of entinostat (**Table 1.4-7**, entry 4) and the aryl amine of **Table 1.4-7**, entry 5. It was subsequently demonstrated to be a more general hydrophobic (or aromatic pi-stacking) interaction and not specific to sulfur, since a phenyl substituent had the same effect (**Table 1.4-7**, entry 6). However, the acetate-release pocket is only able to accommodate one additional ring and the orientation of the ring is critical (in agreement with the early methyl substituents above); a benzothiophene at the 5-position or a thiophene at the 4-position decreases inhibition of HDAC1 and HDAC2 (**Table 1.4-7** entries 7 and 8).

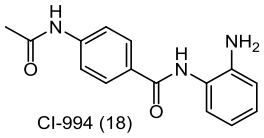
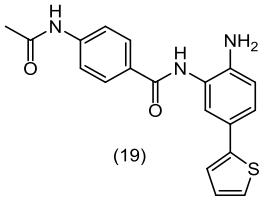
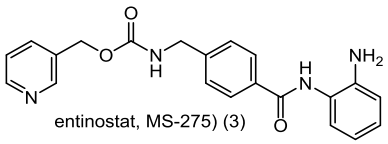
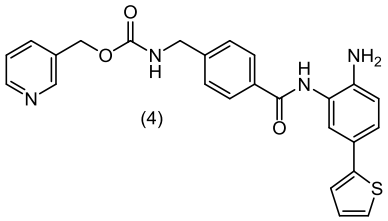
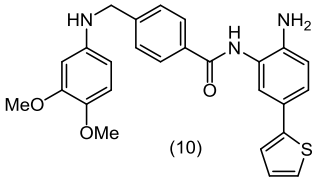
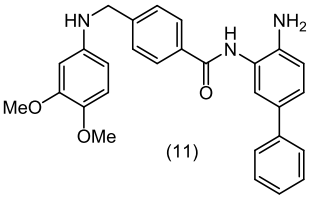
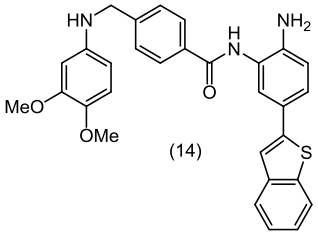
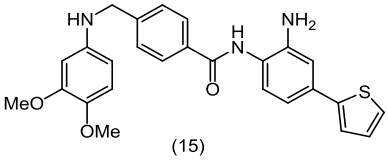
Entry	Compound	HDAC IC_{50} (μM)			
		1	**2**	**3**	**8**
1	 CI-994 (18)	0.9	0.9	1.2	>20
2	 (19)	0.04	0.1	>20	>20
3	 entinostat, MS-275) (3)	0.54	0.5	*	*
4	 (4)	0.02	0.1	*	*
5	 (10)	0.05	0.08	*	*
6	 (11)	0.06	0.1	*	*
7	 (14)	>10	>10	*	*
8	 (15)	>10	>10	*	*

***Table 1.4-7**: Inhibition of HDAC1, 2, 3, and 8 by benzamide inhibitors showing the effect of substituents on the terminal 2-aminophenyl ring, * not determined.*[65]

A thiophene at the 4-position of the 2-aminophenyl ring was detrimental for inhibition of HDAC1 and HDAC2 (**Table 1.4-7** entry 8), and it has recently been found

that in some cases a fluorine at the same position is also detrimental for inhibition of HDAC1 and HDAC2, although to a lesser extent. Importantly inhibition of HDAC3 is unaffected by the introduction of a fluorine atom at the 4-position of the 2-aminophenyl ring, giving rise to increased selectivity for HDAC3 (**Table 1.4-8** entries 2 and 4). However this effect is not generally applicable to all benzamide inhibitors since the 4-fluoro analogue of mocetinostat is no more HDAC3 selective than the parent compound (**Table 1.4-8** entries 5 and 6).

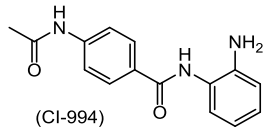
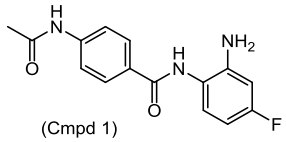
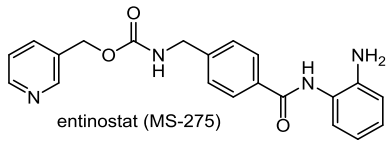
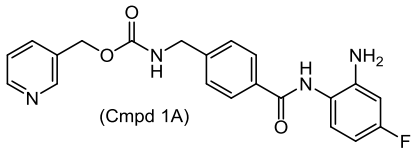
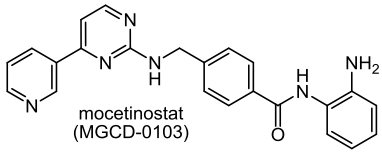
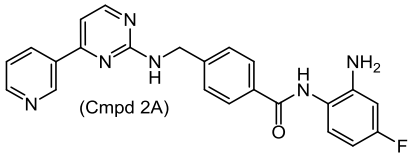
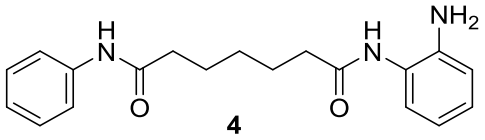
Entry	Compound	HDAC IC_{50} (μM) after 3 h pre-incubation		
		1	2	3
1	 (CI-994)	0.039	0.110	0.038
2	 (Cmpd 1)	1.25	1.42	0.068
3	 entinostat (MS-275)	0.014	0.041	0.034
4	 (Cmpd 1A)	0.120	0.157	0.036
5	 mocetinostat (MGCD-0103)	0.006	0.026	0.023
6	 (Cmpd 2A)	0.012	0.018	0.022

Table 1.4-8*: Inhibition of HDAC1, 2, and 3 by benzamide inhibitors with a 4-fluoro substituent on the terminal 2-aminophenyl ring.*[66]

It is worth noting that inhibition of HDAC11 has not been reported for these HDAC inhibitors with substituents at the 4- or 5-position on the terminal 2-aminophenyl ring, it might have been examined but it has not been reported.

1.4.3 Slow Tight Binding Inhibitors

Certain benzamide inhibitors have been shown to be slow tight-binding HDAC inhibitors, although the mechanism of binding may differ between compounds and between isoforms. This was first reported in 2008 by Chou *et al.* when studying the explanation for the different pharmacological effects of hydroxamic acid and benzamide inhibitors.[67] Initially it was shown that introducing a pre-incubation period in the *in vitro* enzyme assay resulted in changes to the measured IC_{50} value. With the benzamide **4**, inhibition of HDAC1 had reached equilibration after 15 minutes pre-incubation, and this resulted in a 3 fold decrease in IC_{50} value. However for HDAC3 after three hours pre-incubation the IC_{50} had decreased 15 fold (from 5.8 μM to 0.380 μM) and after this time equilibrium had nearly been reached. Investigating the mechanism of slow binding led to the interesting finding that it is different for the two isoforms, HDAC1 follows a single slow step of binding, whereas the mechanism for HDAC3 consists of an initial fast step forming a weakly bound enzyme-inhibitor complex which then undergoes a second slow step such as a conformational change in the structure of the protein.



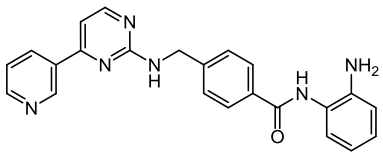
4

***Figure 1.4-6**: Structure of the first benzamide inhibitor determined to be slow tight-binding.*

Despite this publication, a great deal of enzyme inhibition data of benzamide compounds has been published since, without considering the effect of pre-incubation. However in some cases pre-incubation has been considered, and this partly accounts for the sometimes large differences in reported IC_{50} values of compounds. This can be seen in the next section (**Table 1.4-9**) comparing the different IC_{50} values reported for mocetinostat. Some of the data above also highlight this; comparing entries 1 and 3 of **Table 1.4-7** with the same entries of **Table 1.4-8** shows that a 3 hour pre-incubation decreases the IC_{50} value up to 38 fold (entinostat against HDAC1) and also shows that the relative differences between isoforms are not consistent between different structures. This is an added complication when evaluating benzamide HDAC inhibitors *in vitro*, but it can be an advantage *in vivo* because of prolonged effects of histone acetylation as is the case with HDAC3 inhibition in the treatment of Friedreich's ataxia discussed above (section 1.3.2).

1.4.4 Mocetinostat

The lead compound for this project is mocetinostat, also known as MGCD0103, discovered by MethylGene and currently in clinical trials as an anticancer agent.[68] There is a wide range in the IC_{50} values reported for mocetinostat against HDAC1, and also for HDAC2 and HDAC3 although there are fewer reports for these isoforms, the data is summarised below in **Table 1.4-9**. There is only one published IC_{50} value for mocetinostat against HDAC11, contained in the first report from MethylGene. The data shown highlight the variation in reported values for many HDAC inhibitors, particularly benzamide inhibitors because they have a slow on rate and so their inhibition is more sensitive to changes in assay conditions such as pre-incubation time (as discussed above).



Ref.	HDAC IC_{50} (μM)										
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
[66]	0.006	0.026	0.023	*	*	*	*	*	*	*	*
[69]	0.034	0.034	0.998	>10000	*	>10000	>10000	>10000	*	*	*
[70]	0.102	*	*	*	*	>10000	*	*	*	*	*
[71]	0.130	*	0.610	*	*	#32%	*	*	*	*	*
[68]	0.15	0.29	1.66	>10000	>10000	>10000	>10000	>10000	>10000	>10000	0.59
[72]	0.152	*	*	*	*	*	*	*	*	*	*
[73]	0.184	*	*	*	*	*	*	*	*	*	*
[74]	0.20	0.18	*	>10000	>10000	*	>10000	*	*	*	*
[75]	0.82	*	0.62	*	*	>30000	*	>25000	*	*	*
[76]	0.95	0.28	1.67	*	*	*	*	*	*	*	*

***Table 1.4-9**: Published IC_{50} values for mocetinostat. * not determined, # percentage inhibition at 2 μM.*

Extensive SAR analysis of mocetinostat was carried out by MethylGene and subsequently by others, although for many compounds detailed enzyme inhibition data was not published. Some of the compounds in the previous section could be considered mocetinostat analogues in particular those in **Table 1.4-4** and **Table 1.4-8** entry 6. A selection of some other available data will now be briefly discussed, focusing on the inhibition of purified recombinant enzymes.

Entry 2 in **Table 1.4-10** shows the necessity of the methylene unit between the central benzamide ring and the aminopyrimidine, entries 3 and 4 show the location of

the pyrimidine nitrogens are not important and in fact the pyrimidine can be replaced by a phenyl ring to give slightly increased potency. Entries 5-7 demonstrate that the terminal pyridine ring is solvent exposed and can be appended with various groups without loss of activity. Electron donating and withdrawing substituents are tolerated, although some angular bulky groups such as the dimethyl amide (entry 7) give rise to a loss of potency. The pyridine ring can be replaced with a variety of heterocycles, such as thiazole (entry 8), which also maintain potency.

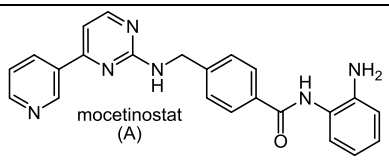
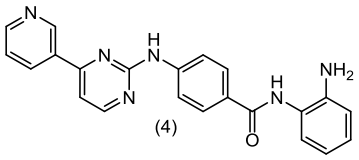
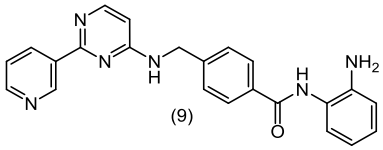
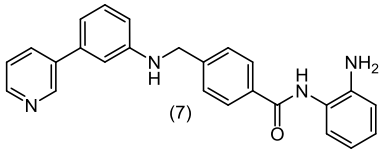
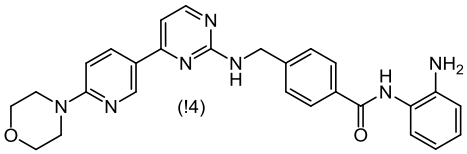
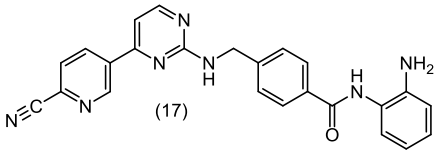
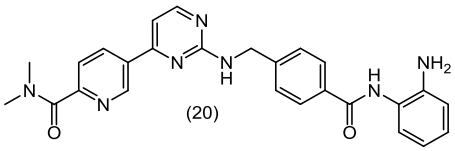
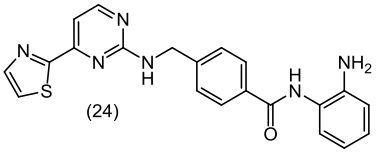
Entry	Compound	HDAC1 IC_{50} (μM)
1	 mocetinostat (A)	0.15
2	 (4)	2.0
3	 (9)	0.20
4	 (7)	0.09
5	 (!4)	0.05
6	 (17)	0.16
7	 (20)	0.42
8	 (24)	0.14

***Table 1.4-10**: Inhibition of recombinant HDAC1by mocetinostat analogues.*[77]

MethylGene summarised their SAR findings in the following diagram (**Figure**

1.4-7), however it only applies to inhibition of HDAC1 since the inhibition data used to generate the SAR was measured against HDAC1. Therefore the effect of different structural modifications on isoform selectivity is not well understood; in addition no chiral mocetinostat analogues have been reported and so the effect of introducing chirality into the cap region remains unexplored.

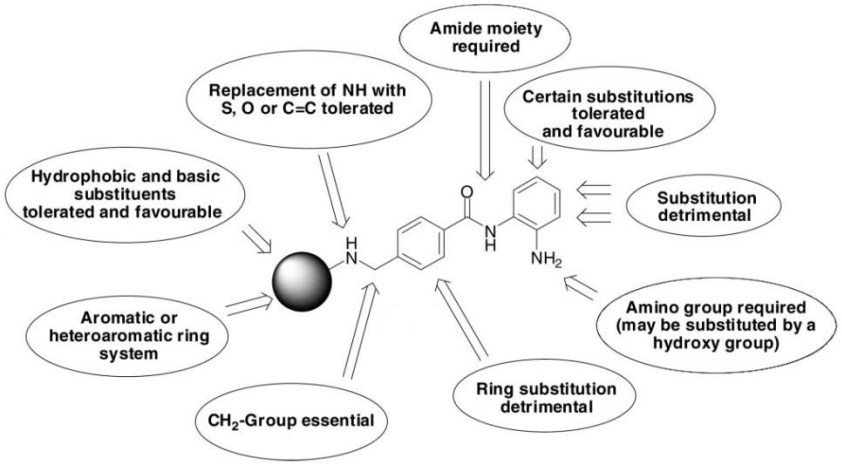


***Figure 1.4-7**: SAR of N-(2-aminophenyl)-4-(arylaminomethyl)benzamides.*[68]

1.4.5 Chiral HDAC inhibitors

The first HDAC inhibitor discovered, natural product TSA (**Figure 1.4-3**), contains a chiral centre in the linker section, but it does not confer much isoform selectivity since TSA inhibits all HDAC isoforms with low nanomolar IC_{50} (except HDAC8; IC_{50} ~0.5 μM). Cyclic depsipeptides such as romidepsin often contain several centres of chirality in the cap group region, but the chiral centres are distant from the entrance to the active site tunnel, so their effect on isoform selectivity is possibly reduced as a result.

Synthetic small molecule HDAC inhibitors incorporating chirality into the cap group were first reported with hydroxamic acid inhibitors and this resulted in the discovery of compounds that were >10 fold selective for HDAC6 over HDAC1 (**Table 1.4-11**). These compounds were derived from phenylalanine as a source of chirality. The limitation of the study is that they have only measured inhibition of two isoforms, and therefore the full selectivity is unknown.

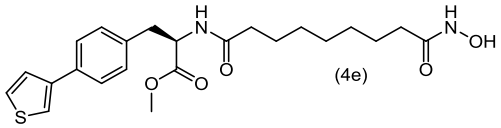
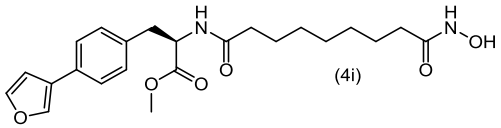
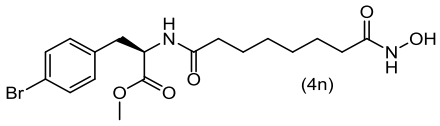
Entry	Compound	HDAC1 IC_{50} (μM)	HDAC6 IC_{50} (μM)	Fold selectivity
1	 (4e)	19.19	1.77	10.8
2	 (4i)	10.3	1.05	9.8
3	 (4n)	22.86	1.69	13.5

Table 1.4-11*: Isoform selective inhibition of immunoprecipitated HDACs by chiral hydroxamic acid inhibitors.*[78]

A similar strategy was employed by Smil *et al.*; a series of hydroxamic acids containing a chiral substituted 3,4-dihydroquinoxalin-2-one cap group was prepared. Of particular note is the discovery that absolute stereochemistry has an effect on isoform selectivity. In the two cases where both pairs of enantiomers were prepared, the *R*-enantiomers both showed far superior HDAC6 selectivity. The most selective compound of the series is the 4-hydroxyphenyl compound derived from tyrosine, it has an impressive selectivity for HADC6 (41 fold versus HDAC2 and 91 fold versus HDAC8) similar selectivity against other isoforms was stated but the data was not shown.

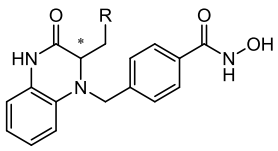
		HDAC IC_{50} (μM)			HDAC6: HDAC2 selectivity	HDAC6: HDAC8 selectivity
Entry	**R**	**2**	**6**	**8**		
1	(*R*)-phenyl (7b)	0.26	0.01	0.53	26	53
2	(*S*)-phenyl (7c)	0.46	0.22	0.21	2	1
3	(*R*)-2-thienyl (7d)	0.87	0.04	0.69	21	17
4	(*S*)-2-thienyl (7e)	1.48	0.31	1.19	5	4
5	(*R*)-*p*-OHC_6H_4 (7g)	0.33	0.01	0.73	41	91

Table 1.4-12*: Inhibition of affinity purified recombinant HDACs. Selectivity against HDAC1, 3, 4, 5, and 7 was similar to HDAC 2 and 8.*[79]

Another example of chiral inhibitors being tested against many HDAC isoforms is that of two α-oxime amides, prepared from glutamic acid as a source of chirality.

However the compounds are only weak inhibitors, and not particularly selective (**Table 1.4-13**) demonstrating that introducing chirality does not always increase selectivity.

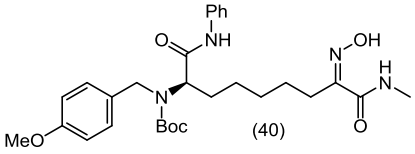
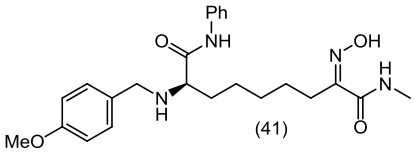
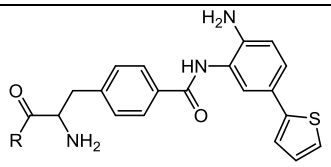
Entry	Structure	HDAC IC_{50}s (μM)								
		1	**2**	**3**	**4**	**5**	**6**	**8**	**9**	**10**
1	 (40)	81.1	>100	49.4	>100	76.7	64.2	>100	>100	98.5
2	 (41)	17.5	95.9	10.6	59.2	45.8	28.7	94.6	65.7	37.8

***Table 1.4-13**: Inhibition of human HDAC isoforms.*[53]

Chiral benzamide HDAC inhibitors have also been described; in the first report the absolute stereochemistry does not seem to have much effect on inhibition of HDAC1. Furthermore the effect on selectivity remains unknown since the homochiral compounds were only tested against HDAC1. One racemic analogue demonstrated high levels of selectivity for HDAC1, however it is presumed that this selectivity arises from the presence of the thienyl substituent at the 5-position on the terminal 2-aminophenyl ring because the short, zinc-binding group that doesn't contain the chiral centre (**Table 1.4-14**, entry 6) also demonstrated similar selectivity (this was also discussed previously in **Table 1.4-7**).



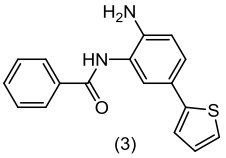
Entry	Structure	HDAC IC_{50}s (μM)								
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**11**
1	R=NH_2 (12)	0.010	0.130	3.4	>50	>50	>50	>50	35	3.6
2	R=NH_2 (*R*) (16)	0.012	*	*	*	*	*	*	*	*
3	R=NH_2 (*S*) (17)	0.011	*	*	*	*	*	*	*	*
4	R=NHMe (*R*) (18)	0.014	*	*	*	*	*	*	*	*
5	R=NHMe (*S*) (19)	0.022	*	*	*	*	*	*	*	*
6	 (3)	0.048	0.362	*	*	*	*	*	*	*

***Table 1.4-14**: Inhibition of immunoprecipitated HDACs. * not determined.*[80]

In a more recent example the influence of absolute configuration is more compelling, however there is not clear data linking changes of absolute stereochemistry and differences to *in vitro* enzyme inhibition, or isoform selectivity.[81] In contrast a cellular differentiation assay is used to compare the stereoisomers, and it does show that changes to the absolute configuration can have a big impact on the amount of differentiation induced (Figure 1.4-8). For example the two most potent compounds share the same 2-(*S*), 3-(*R*) stereochemistry, which is interesting because these are the two positions that are closest to the HDAC tunnel. The exocyclic chiral centre C_1 generally did not exert as much influence on the compound's ability to induce differentiation. HDAC enzyme inhibition data is only given for one compound (BRD8430) and it demonstrates some moderate selectivity for HDAC1 versus HDAC2 and HDAC3, and good selectivity versus HDAC4, 5, 6, 7, 8, and 9 (**Table 1.4-15**).

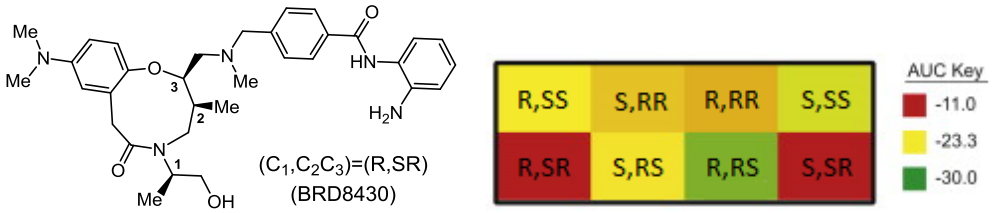


Figure 1.4-8: Structure of BRD8430, and a heat map showing area under the curve (AUC) for different stereochemical variants. AUC was calculated from a plot of differentiation score against concentration, having measured a differentiation score at several concentrations.[81]

		HDAC IC_{50}s (µM)								
Entry	**Structure**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
1	(R,SR) - BRD8430	0.069	0.56	1.3	>20	>20	>20	>20	>20	>20

***Table 1.4-15**: Inhibition of recombinant HDACs.*[81]

1.5 HDAC enzyme assays

The first HDAC assay was based on a radiolabelled substrate, chicken reticulocyte histones acetylated with tritium labelled acetate.[82] After incubation of the HDAC enzyme with the radiolabelled histones, an extraction procedure, centrifugation and addition to a liquid scintillation cocktail, the radioactivity can be measured. However the production of the radiolabelled acetylated histone substrate is complicated and batch to batch variation means that standardisation of the assay is difficult.[83] Added to this, the complexity of the procedure and disadvantages of using radioactivity (personal exposure, equipment decontamination and waste disposal) meant that non-radioactive assays were sought.

The first non-radioactive HDAC substrate for assays was the fluorescent substrate Boc-Lys(Ac)-AMC (MAL), that is deacetylated by HDAC enzymes to give Boc-Lys-AMC (ML) (**Figure 1.5-1**), which has the same fluorescence properties as MAL.[84,85] Therefore to develop the substrate for use in an assay, an extraction procedure was required and reverse phase HPLC used to separate MAL and ML which were quantified by fluorescence detection. This meant that this assay was also labour intensive and not suited to high throughput, so an improved extraction procedure was developed which enabled use of a plate reader to measure fluorescence and quantify the extent of deacetylation.[86]

However a truly homogenous assay without any extraction procedure was subsequently developed; a derivatisation reagent, naphthalene-(2,3)-dicarboxaldehyde, is added after the HDAC reaction and it reacts with the deacetylated product (ML).[87] The amount of remaining substrate MAL can be quantified without any extraction since the fluorescence of naphthyl-ML product **5** is quenched.

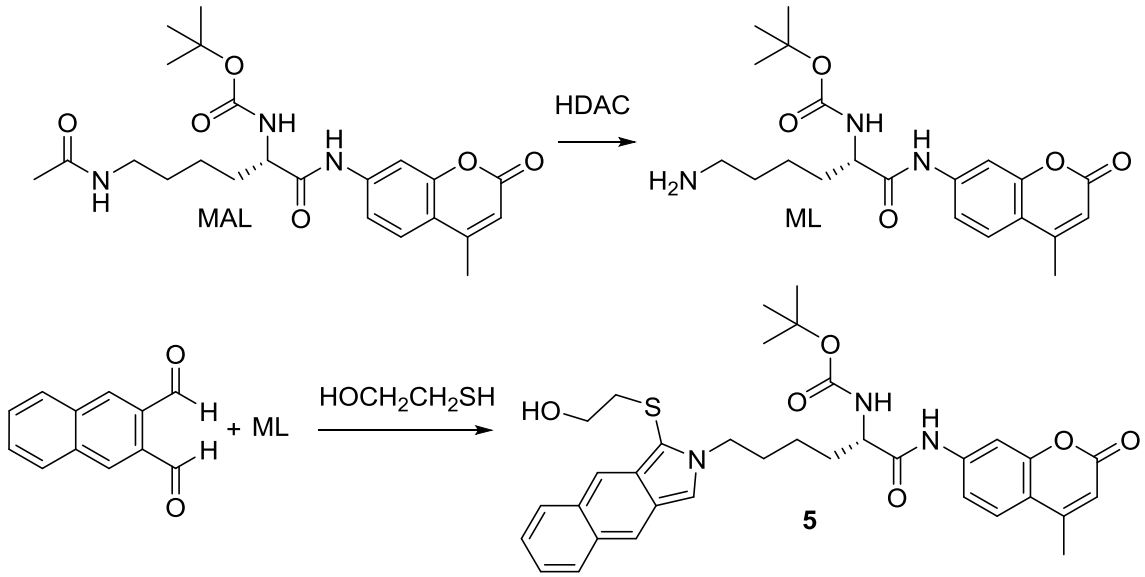


***Figure 1.5-1**: Deacetylation of MAL to give ML and conjugation with naphthalene dicarboxaldehyde.*

At the same time an alternative homogeneous assay was developed which made use of an enzyme in the second step.[88,89] Trypsin, a proteolytic enzyme, selectively reacts with ML to release the coumarin fluorophore (AMC) which has a different wavelength of excitation and emission so can be quantified without separation. This assay had originally been developed using tripeptide and tetrapeptide substrates, but it is also effective using MAL (although higher trypsin concentrations are required) and there are also commercial assay kits that operate using this same principle,[90] see section

5.1 (p 96) for more detail.

Deacetylase activity can also be measured by detecting the acetate produced from deacetylation of an acetylated substrate. There are many ways in which the acetate can be detected, firstly by a complicated coupling of other enzymatic reactions (**Figure 1.5-2**); acetic acid can be converted into acetyl-coenzyme A (acetyl-CoA) by acetyl-CoA synthase (ACS), then this can be used to convert oxaloacetate into citrate by citrate synthase (CS). The oxaloacetate is made from malate using malate dehydrogenase (MDH); this process also converts NAD^+ to NADH, and NADH can be monitored by absorption at 340 nm (**Figure 1.5-2**).[91] In another assay a trifluoroacetylated substrate gives trifluoroacetic acid upon deacetylation, and this can be measured directly using ^{19}F NMR to quantify the extent of deacetylation, but the substrate is not a good mimic of a natural acetylated lysine.[92] Likewise even ^1H NMR can be used to monitor the production of acetic acid from a non-labelled acetylated substrate based on p53.[93] However these NMR assays require relatively large amounts of enzyme to function correctly.

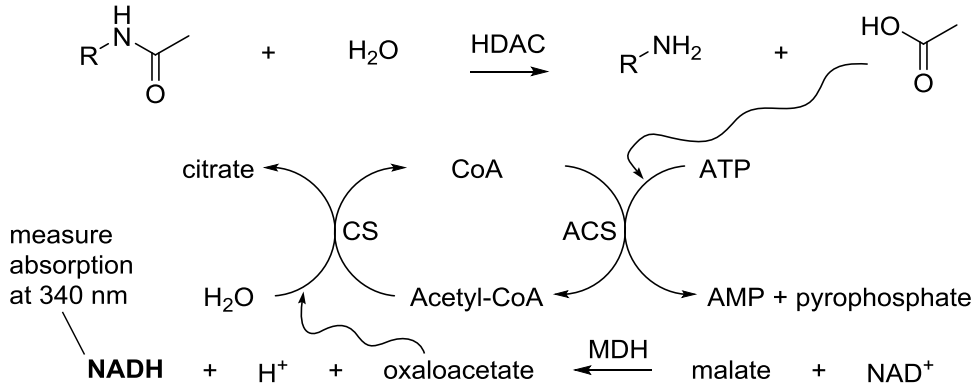


***Figure 1.5-2**: Enzyme linked measurement of acetic acid production.*[91]

The assays discussed so far, are all end-point assays (except the NMR assays), that is they measure the amount of deacetylation at the end of the assay, and so they are not very useful for measuring kinetic values. One such assay that has been developed in order to measure off rates of inhibitors is a displacement assay, where a fluorescent ligand (coumarin-SAHA) is added in large excess concentration to the bound inhibitor and HDAC enzyme mixture.[94] During the course of the assay coumarin-SAHA outcompetes the previously bound inhibitor, and the coumarin SAHA has a lower intensity of emission when bound to the HDAC enzyme because of fluorescence quenching (**Figure 1.5-3**). The change in the intensity of the emission is proportional to the amount of coumarin-SAHA bound to the enzyme and this correlates with the displacement of bound inhibitor. However this assay only studies inhibitor binding, not

actual enzyme inhibition (although the two should correlate since the inhibition is competitive) and it is also limited by the affinity of the coumarin-SAHA reporter molecule's binding to the HDAC enzyme. Furthermore the small difference in fluorescence intensity gives a relatively low signal-to-noise ratio.

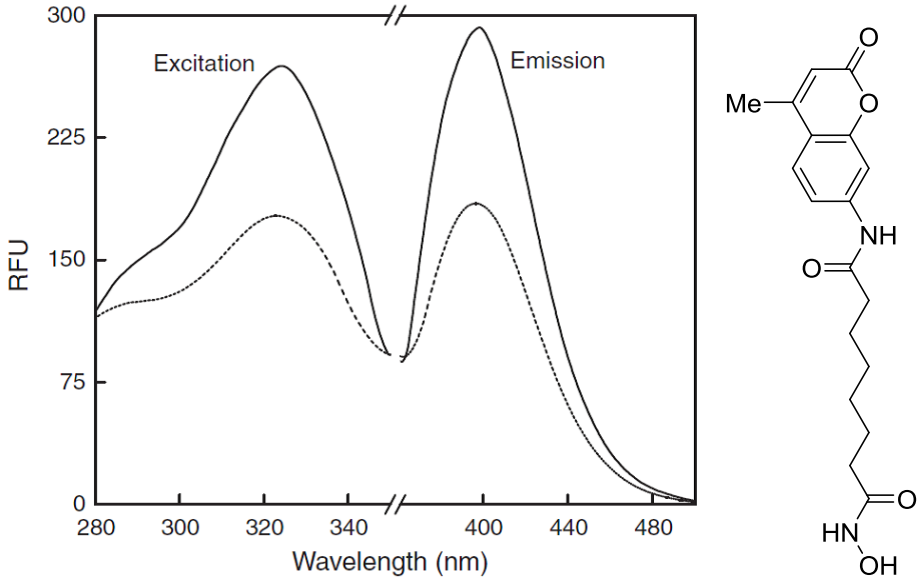


***Figure 1.5-3**: Structure of coumarin-SAHA and its excitation and fluorescence emission spectra of the free form (solid line) and when enzyme bound (dotted line).*[94]

Another assay has been reported that measures time dependent inhibition, and is used to quantify the kinetics of inhibitor binding. Furthermore, the assay can be used to determine the mechanism of slow tight-binding that is observed with benzamide HDAC inhibitors and certain isoforms (as mentioned above, section 1.4.2, p 29).[67] This assay is a continuous monitoring assay but instead of measuring inhibitor binding, it measures enzymatic activity. The main concept of the assay is the same as the fluorescent assay in which trypsin is used to develop the fluorescent signal. Instead of adding trypsin after the deacetylation reaction, a more selective enzyme, endoproteinase Lys-C, is used together with the HDAC enzyme during the deacetylation reaction. In that way, as soon as the deacetylated substrate is produced, the fluorophore AMC is cleaved and then fluorescence can be measured continuously during the deacetylation reaction (see section 5.2 (p 102) for a more detailed discussion).

Recently the development of mobility shift assay technology has enabled the rapid high throughput evaluation of HDAC inhibitors using a fluorescent assay without a coupled second step. In addition this technology allows the measurement of kinetic values, and does not require large amounts of protein sample. PerkinElmer's technology is based upon 'sipping' extremely small volumes of a reaction mixture (down to 10 nL),

then LabChip technology separates the acetylated substrate and deacetylated product by electrophoresis and quantifies the relative amounts of both by LED induced fluorescence. This technology was originally used for evaluating inhibition of kinase enzymes but the Broad Institute have developed substrates that enable it to be applied to HDAC enzymes.[95]

All of the above assays can be carried out with different sources of HDAC activity; either cell extracts from cells containing a mixture of HDAC isoforms, immunoprecipitated HDAC isoforms which are still in the native multi-protein complexes, or purified recombinant HDAC isoforms. Isoform selectivity can be measured when using individual isoforms by calculating an IC_{50} value for each isoform. Recently a novel assay was developed in which isoform selectivity could be determined using a native mixture of HDAC isoforms obtained from cell lysates. It is a chemoproteomics based assay and so it does not have the same levels of throughput as the fluorescent assays above, however it gives a more accurate reflection of the *in vivo* isoform selectivity profile.[96] Two matrix immobilised probe molecules bind to the HDAC enzyme complexes (at the active site), these complexes can then be pulled out of the solution using the attached matrix. The proteins in the complexes are quantified by digesting with trypsin and then isobaric tagging and tandem mass spectrometry. By adding different concentrations of inhibitors to the incubation of probe molecules and cellular lysate, the inhibitor displaces the probe and the resulting reduction of individual protein levels can be measured. This gives a measure of the relative binding strengths of the inhibitor towards each HDAC complex and therefore allows one to calculate the isoform selectivity profile.

2 Inhibitors with occupancy of the acetate-release pocket

The acetate-release pocket has previously been studied by those seeking to design improved HDAC inhibitors as discussed in section 1.4.2 (**Table 1.4-7** and **Table 1.4-8**). However in many of these cases compounds have only been tested against one isoform, and so there remains a possibility that these compounds could have been selective inhibitors of an isoform that was not tested against. Furthermore a homology model of HDAC11 (supplied by GSK) containing mocetinostat docked into the active site suggested a novel and unexpected binding pose in which the pyridine cap group is buried deep within the acetate-release pocket of the enzyme (**Figure 1.5-1**). This mode of binding is a reversal of the standard pharmacophore model (**Figure 1.4-1**, p 17); dockings of known HDAC inhibitors in other HDAC isoforms show the aromatic cap group binding to the exterior surface of the protein. The validity of this homology model and mocetinostat docking is unknown, and since HDAC11 is the most recently identified HDAC (see section 1.1, p 12) there are few reports in the literature of HDAC11 inhibition data. Likewise there are no crystal structures available for HDAC11 in the protein binding database, and no other dockings reported in the literature; so it is not easy to assess the accuracy of the GSK model.

a) *b)*

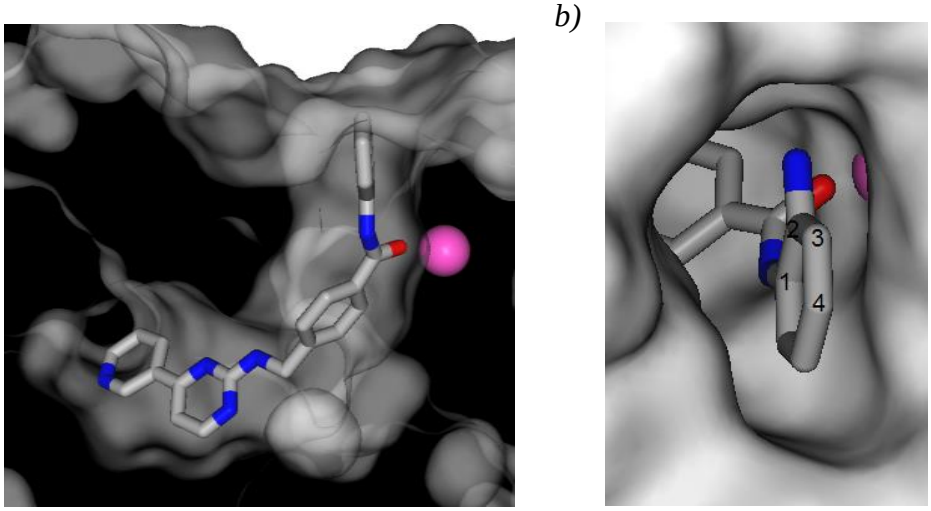
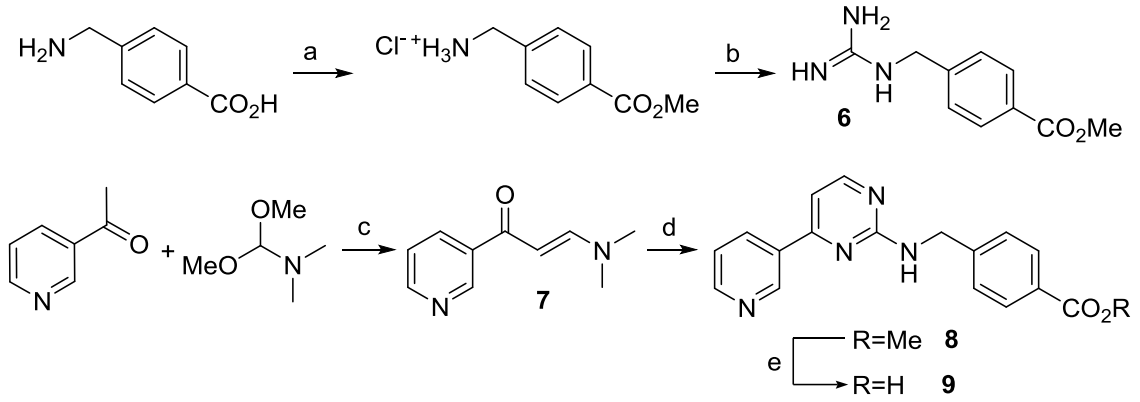


***Figure 1.5-1**: Images showing mocetinostat (stick) docked into HDAC11 model (Zn^{2+} ion in pink). a) Cut away of the cavity showing proximity of the benzamide to the surface. b) View of the cavity from the protein exterior showing that 4-substituted benzamides could be tolerated.*

The observed reverse binding pose implies that the benzamide group is close to the surface of the protein and the 4-position (*para* to the amide) is solvent exposed (**Figure 1.5-1**). This led us to believe that substitution at the 4-position of the 2-aminophenyl ring could give rise to compounds that were still active inhibitors of HDAC11. If this hypothesis were correct then the resulting compounds could be selective inhibitors, since it is known that substitution at the 4-position is detrimental for inhibition of HDAC1 and HDAC2 (**Figure 1.4-7**, p 32). So in order to investigate the accuracy of the GSK docking model, and to explore whether substitution *para* to the amide could be a means of enhancing the isoform selectivity of mocetinostat, a series of these analogues was synthesised.

2.1 Synthesis of 4-substituted *N*-(2-aminophenyl)benzamides

It is likely that the electronics of the *N*-(2-aminophenyl)benzamide are important for successful inhibitor binding since both the carbonyl oxygen and the aniline nitrogen atoms are involved in coordinating the zinc ion. Therefore mainly substituents that are largely electronically neutral were targeted but for completeness some examples of strongly electron donating and electron withdrawing substituents were also sought. Some polar and some non-polar groups were chosen as were a range of different sized groups. The synthesis of these compounds is now discussed.

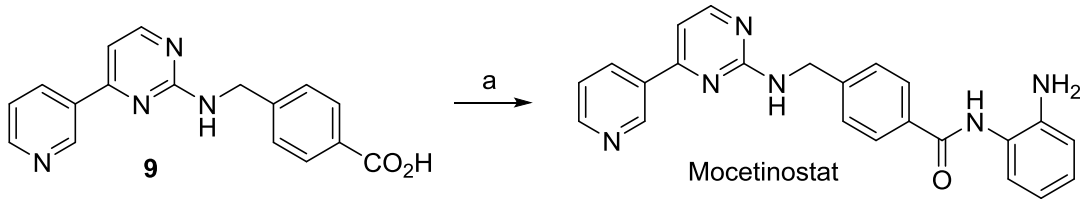


***Scheme 2.1-1**: a) $SOCl_2$, MeOH, reflux, 3 h, 85%; b) 1H-pyrazole-1-carboxamidine hydrochloride, EtOH, refux, 3 h, 80%; c) reflux, 5 h, 83%; d) **6**, propan-2-ol, reflux, 20 h, 67%; e) LiOH.H_2O, THF, H_2O, rt, 24 h, 97%.*

Benzoic acid **9** (**Scheme 2.1-1**) was synthesised according to a literature procedure;[68] condensation of 3-acetylpyridine and dimethylformamide dimethylacetal gave the propenone **7**. Reaction of methyl-4-(aminomethyl)benzoate hydrochloride and 1*H*-pyrazole-1-carboxamidine hydrochloride gave guanidine **6**, which was subsequently

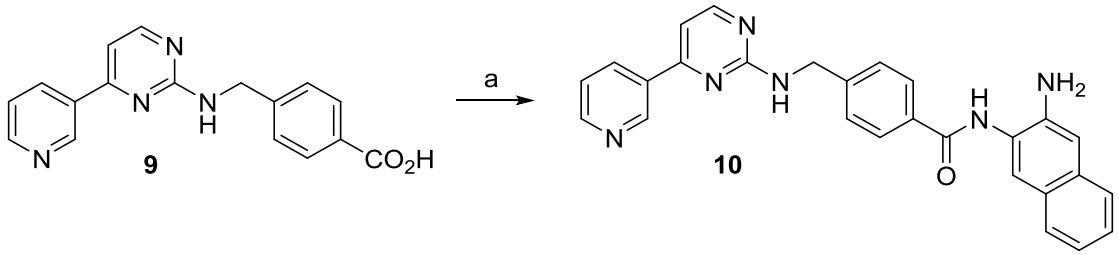
condensed with enone **7** to give methyl ester **8**. Saponification of the methyl ester using lithium hydroxide gave the carboxylic acid **9**, ready for coupling with 4-substituted anilines.

The parent compound mocetinostat was made in 59% yield from a BOP-mediated coupling of **9** with phenylene-1,2-diamine.



Scheme 2.1-2*: a) phenylene-1,2-diamine, BOP, DMF, rt, 24 h, 59%.*

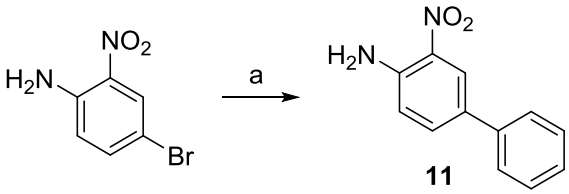
In order to synthesise the naphthyl-substituted analogue, carboxylic acid **9** was first activated as the acid chloride by reaction with thionyl chloride, and then reacted with naphthalene-2,3-diamine to give the product **10** in 32% yield.



Scheme 2.1-3*: a) i) $SOCl_2$, toluene, reflux, 3 h.*
ii) naphthalene-2,3-diamine, NEt_3, DCM, DMF, 0 °C-rt, 1.5 h, 32%.

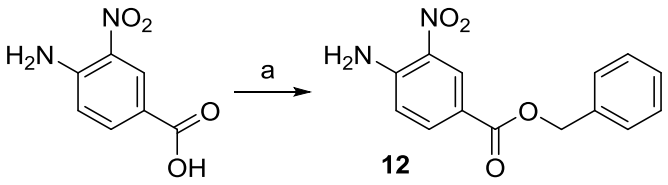
In the case of non-symmetrical examples the diamine could not be coupled directly as this would give a mixture of regioisomers, therefore 4-substitued-2-nitroanilines were coupled with the carboxylic acid **9**, followed by reduction of the nitro group. The nitroanilines were prepared as follows.

A Suzuki coupling of 4-bromo-2-nitroaniline and phenylboronic acid afforded the 4-phenyl nitroaniline **11** in 56% yield.



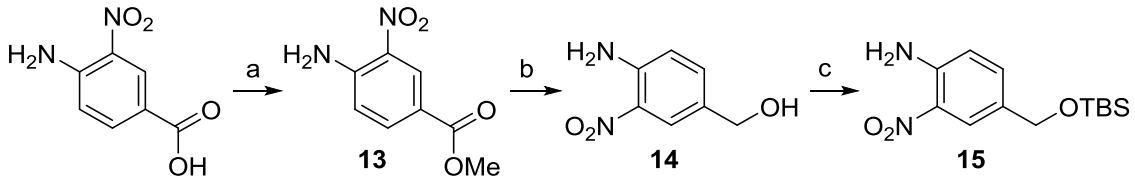
Scheme 2.1-4*: a) $PhB(OH)_2$, $Pd(PPh_3)_4$, Na_2CO_3, 1,2-dimethoxyethane, H_2O, 80 °C, 48 h, 56%.*

Protection of 4-amino-3-nitrobenzoic acid as a benzyl ester was readily achieved in near quantitative yield by treating with benzyl bromide and potassium carbonate in DMF.



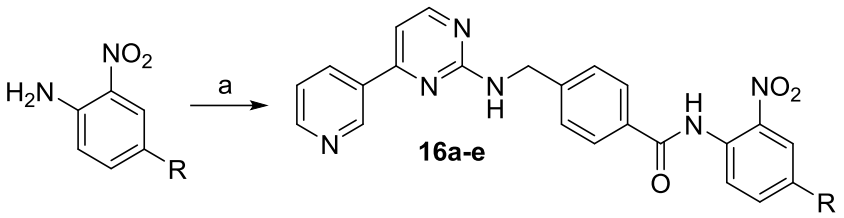
***Scheme 2.1-5**: a) benzyl bromide, K_2CO_3, DMF, rt, 18 h, 96%.*

For the 4-hydroxymethyl analogue; reduction of the carboxylic acid of 4-amino-3-nitrobenzoic acid was attempted using sodium borohydride and iodine but the reaction only returned starting materials. So the methyl ester **13** was prepared and reduction attempted using sodium borohydride in both THF and ethanol but both gave complex mixtures and incomplete reactions. Successful reduction of methyl ester **13** was achieved using DIBAL to give alcohol **14** which was subsequently protected as a *tert*-butyldimethylsilyl ether by reaction with *tert*-butyldimethylsilyl chloride and imidazole.



***Scheme 2.1-6**: a) $SOCl_2$, MeOH, reflux, 16 h, 94%; b) DIBAL, DCM, THF, 0 °C for 1 h, rt for 3 h, 98%; c) TBSCl, imidazole, DMF, rt, 16 h, 89%.*

Conditions for coupling such highly deactivated nitroanilines, which are very non-nucleophilic were found in the literature; BOP coupling reagent and sodium hydride (which activates the nitrogen as a sodium amide anion).[97] Even using these conditions the couplings proceeded in low yield, and therefore in some cases couplings were attempted with the acid chloride of **9** having used sodium hydride to deprotonate the nitroaniline, but these were also low yielding (**Table 2.1-1**). A variety of nitro reduction methods were attempted although in most cases catalytic hydrogenation with palladium on activated carbon was most successful. A summary of the reactions can be found in **Table 2.1-2**, it is worth noting that hydrogenation of **16e** did consume starting materials, however purification of the product was unsuccessful.

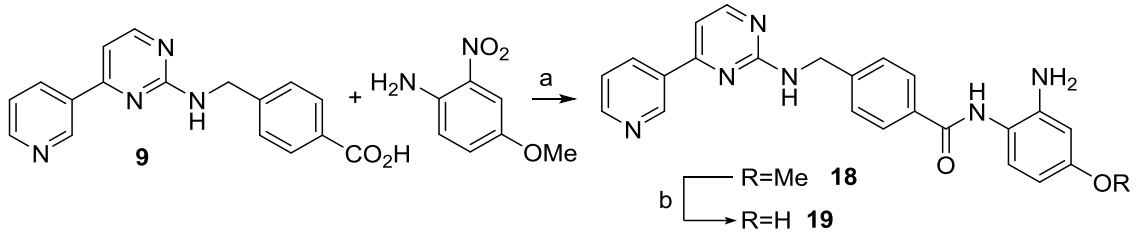


***Scheme 2.1-7**: See **Table 2.1-1** below for conditions.*

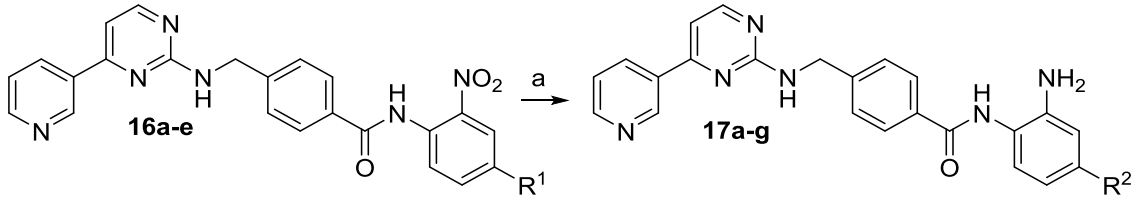
	R	a)
16a	Br	**9**, BOP, pyridine, then sodium hydride, rt 2.5 h, 70%.
16b	Ph	**9**, BOP, pyridine, then Sodium hydride, rt, 7 h, 39%.
16c	CO_2Bn	Sodium hydride, anh. pyridine, −10 °C, 5 min; then acid chloride*, rt, 2 h, 51%.
16d	CH_2OTBS	**9**, BOP, pyridine, then sodium hydride, rt, 2.5 h, 41%.
16e	CN	Sodium hydride, anh. pyridine, −10 °C, 10 min; then acid chloride*, 0 °C, 5 h, 44%.

***Table 2.1-1**: Conditions for amide coupling.*Preparation of acid chloride: **9**, $SOCl_2$, toluene, reflux, 3 h.*

For the synthesis of a strongly electron donating analogue such as the 4-hydroxy substituent, the amide coupling reaction is sufficiently regioselective, because of the enhanced nucleophilicity of the nitrogen *para* to oxygen. In this way BOP mediated coupling of 4-methoxy-1,2-phenylene diamine with benzoic acid **9** gave the desired product **18** in 70% yield. Demethylation using boron tribromide then afforded the 4-hydroxy substituted compound **19**.



***Scheme 2.1-8**: a) BOP, DMF, rt, 27 h, 70%; b) BBr_3, DCM, rt, 24 h, 50%.*

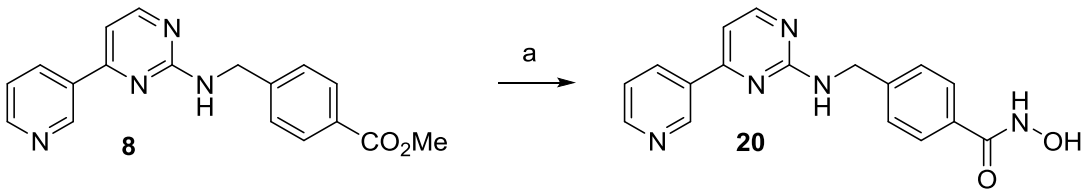


***Scheme 2.1-9**: See **Table 2.1-2** below for conditions.*

	R^1	R^2	Successful conditions	Unsuccessful attempts
17a	Br	Br	$FeSO_4·7H_2O$, aq. NH_3, EtOH, 60 °C, 4 h, 61%.	$SnCl_2·2H_2O$
17b	Ph	Ph	H_2, 10% Pd/C, MeOH, DCM, rt, 6 h, 31%.	$FeSO_4·7H_2O$, aq. NH_3
17c	CO_2Bn	CO_2Bn	H_2, 10% Pd/C, MeOH, DCM, rt, 5.5 h, 22%.	-
17d	CH_2OTBS	CH_2OTBS	H_2, 10% Pd/C, MeOH, $CHCl_3$, rt, 7 h, 36%.	-
17e	CN	CN	-	H_2, 10% Pd/C, MeOH, rt, 7 h.
17f	CO_2Bn	CO_2H	H_2, 10% Pd/C, MeOH, DCM, rt, 45 h, 24%.	-
17g	CH_2OTBS	CH_2OH	$SnCl_2·2H_2O$, NH_4OAc, 1:1:1 THF/MeOH/H_2O, rt, 21 h, then 50 °C, 1 h, 46%.	-

***Table 2.1-2**: Conditions for nitro reduction.*

In order to verify that it was the *N*-(2-aminophenyl)benzamide moiety that conferred both isoform selectivity, and slow tight-binding behaviour (see section 5.2) to mocetinostat, the hydroxamic acid analogue was synthesised. Formation of the hydroxamic acid **20** directly from methyl ester **8** proceeded as expected and gave the desired product in 42% yield.



***Scheme 2.1-10**: a) hydroxylamine (50% aq.), NaOH, THF, 0 °C for 10 min, rt for 5 min; 42%.*

2.2 Enzyme assay results

The series of 4-substituted mocetinostat analogues were evaluated for inhibition of HDAC2, HDAC3-NCoR1, HDAC6 and HDAC8 (**Table 2.2-1**). However a reliable assay against HDAC11 that was able to reproduce the literature IC_{50} value of mocetinostat (or even provide a sigmoidal percentage inhibition against $\log_{10}$(concentration) curve) could not be developed (see section 5.1, p 96). This meant that the accuracy of both the GSK docking model and the hypothesis regarding HDAC11 selective inhibitors could not be established.

Of the series, 4-methoxy and 4-hydroxy substituents (**18** and **19**) were most potent against HDAC2 and HDAC3-NCoR1, although both were less potent than mocetinostat. For both isoforms, the 4-methoxy substituent conferred some 6-fold greater potency than the 4-hydroxy compound. The maintained inhibition of HDAC2 and HDAC3-NCoR1 for **18** and **19** in this series may reflect a stronger binding to zinc arising from increased electron density provided by the mesomeric effect of the methoxy and hydroxy groups, respectively. However it is likely that a combination of several factors influence the differences in inhibition across the series, including both the steric demands in the acetate-release pocket and the polarity of the substituent.

An aminonaphthyl terminus (**10**) conferred good selectivity (30 fold) for HDAC3-NCoR1 over all other isoforms tested, including HDAC1 (>20 μM, not shown in table). In contrast, a 4-phenyl derivative (**17b**) showed no inhibition, suggesting that there is a strict limit on the length of the 4-substituent to retain HDAC3 inhibition. However despite this increase in selectivity the aminonaphthyl containing compound **10** was ~30 fold less potent than mocetinostat against HDAC3-NCoR1 and so this terminal ring was not investigated any further. Since investigation of this series of compounds did not disclose a substituted *N*-(2-aminophenyl)benzamide unit of improved potency, the binding moiety present in mocetinostat was used in further studies.

As expected hydroxamic acid **20** lacks the same isoform selectivity as mocetinostat, it also inhibits HDAC6 and HDAC8 (**Table 2.2-1**), confirming that the origin of mocetinostat's isoform selectivity is the benzamide zinc-binding group.

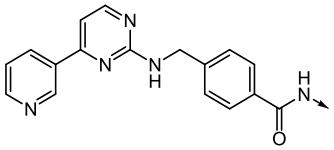
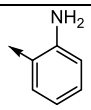
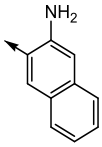
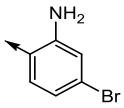
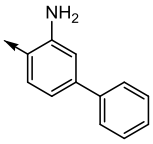
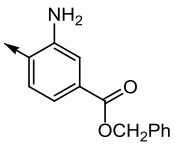
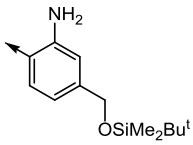
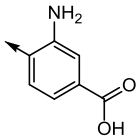
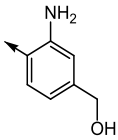
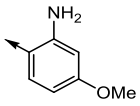
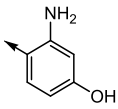
		HDAC inhibitory activity IC_{50} (μM)			
No.	**Structure**	**HDAC2**	**HDAC3-NCoR1**	**HDAC6**	**HDAC8**
mocetinostat		0.022±0.003	0.022±0.001	>20	35.0±4.4
10		>20	0.63±0.011	>20	>20
17a		>20	4.72±0.13	>20	>20
17b		16.7±7.2	>20	>20	>20
17c		>20	>20	>20	>20
17d		>20	>20	>20	>20
17f		>20	>20	>20	>20
17g		>20	>20	>20	>20
18		0.248±0.023	0.111±0.029	>20	>20
19		1.43±0.20	0.719±0.065	>20	>20
20		0.219±0.027	0.031±0.001	0.042±0.019	0.41±0.1

Table 2.2-1*: Inhibition of purified recombinant HDAC enzymes. >20 less than 45% inhibition at 20 μM. Data is from duplicate experiments and ± standard error measurement of the IC_{50} determination.*[98]

2.3 Conclusion

In summary, after careful optimisation of the synthetic procedures, it was possible to readily prepare a set of substituted mocetinostat analogues in moderate overall yield. Analogues with groups at the 4-position of the aminophenyl ring were targeted in order to test an *in silico* docking result and to measure individual HDAC isoform SAR previously unreported for mocetinostat derivatives. The substituents of analogues made, possessed a large variety of different properties and thus the compounds probed the acetate-release pocket in numerous ways, this included; size, shape, polarity, mesomeric and inductive effects, and hydrogen bonding ability. Unfortunately due to the unavailability of a reliable assay for inhibition of HDAC11 (see section 5.1), verification of the *in silico* model was not possible. Additionally inhibition of the other isoforms tested was significantly weaker for all substituted analogues compared to inhibition by the parent, unsubstituted, mocetinostat. Therefore the unsubstituted terminal aminophenyl ring was retained for future work.

3 Chiral 5-membered heterocyclic capping groups

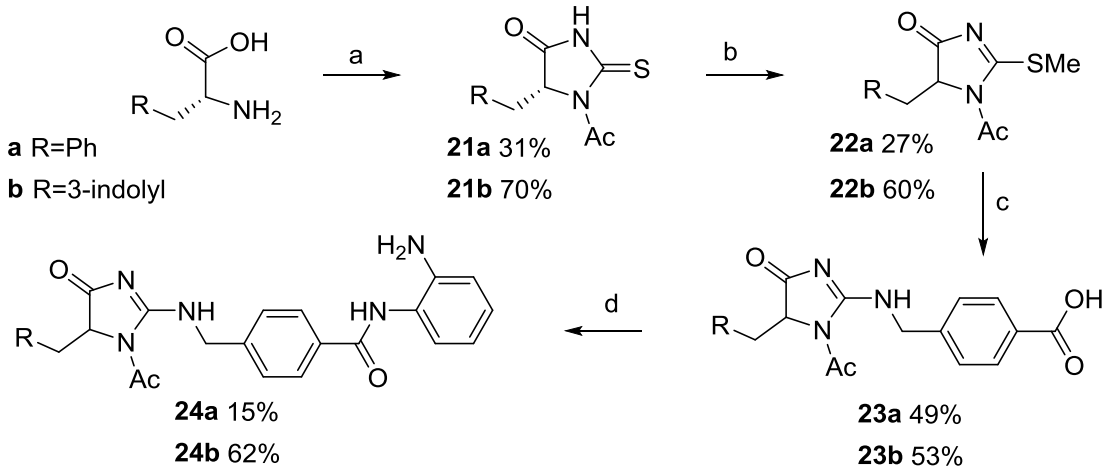
Chiral capping groups have been shown to increase isoform selectivity in hydroxamic acid HDAC inhibitors[79] but have not been thoroughly investigated in conjunction with the benzamide zinc-binding group. This chapter describes the synthesis and evaluation of several series of compounds that are analogues of mocetinostat in which the pyrimidine ring has been replaced by a three-dimensional, chiral heterocycle. Heterocycles were chosen that would match the location of heteroatoms in the heterocycles with the nitrogen atoms in the aminopyrimidine. The heterocycles were designed to mimic the overall shape of the substituted pyrimidine in mocetinostat, whilst at the same time being readily accessible from α-amino acids. The α-amino acids would be used as chiral building blocks to provide a source of chirality.

3.1 Synthesis of dihydroimidazol-4-ones

3.1.1 *N*-acetyl dihydroimidazolones

The inhibitors were prepared according to the route initially developed by Yiannaki (**Scheme 3.1-1**).[98] First, D-phenylalanine was heated to 100 °C with ammonium thiocyanate in acetic anhydride to form the 2-thiohydantoin ring **(*R*)-21a** in 31% yield; similarly, D-tryptophan gave **(*R*)-21b** in 70% yield.[99] These 2-thiohydantoins were subjected to reported *S*-methylation conditions, potassium carbonate and iodomethane in acetonitrile at room temperature for 2.5 h,[100] to give the corresponding methylated products **22a**/**22b** in 27% and 60% yield respectively. During this reaction racemisation of the dihydroimidazolone occurs, driven by formation of a heteroaromatic enolate. However since this racemisation was not detected until the end of the route (optical rotation measurements were waiting for a polarimeter to be fixed), no attempt was made to minimise racemisation by optimising conditions of the reaction. Instead alternative heterocycles were targeted in later work (see section 3.1.3).

Displacement of methanethiol from **22a**/**22b** by 4-(aminomethyl)benzoic acid gave the benzoic acids **23a**/**23b**; isolated in moderate yield (49% and 53% respectively) simply by filtration of the cooled reaction mixture. The BOP-mediated coupling of these acids with phenylene-1,2-diamine gave several impurities, but the benzamides **24a**/**24b** were isolated in 15% and 62% yield respectively, after purification.



***Scheme 3.1-1**: a) NH_4SCN, Ac_2O, 100 °C, (for **(R)-21a**: 1 h) (for **(R)-21b**: 10 min); b) K_2CO_3, iodomethane, MeCN, rt, 2.5 h; c) 4-(methylamino)benzoic acid, EtOH, reflux, 18 h; d) BOP, NEt_3, phenylene-1,2-diamine, DMF, rt, 3.5 h.*

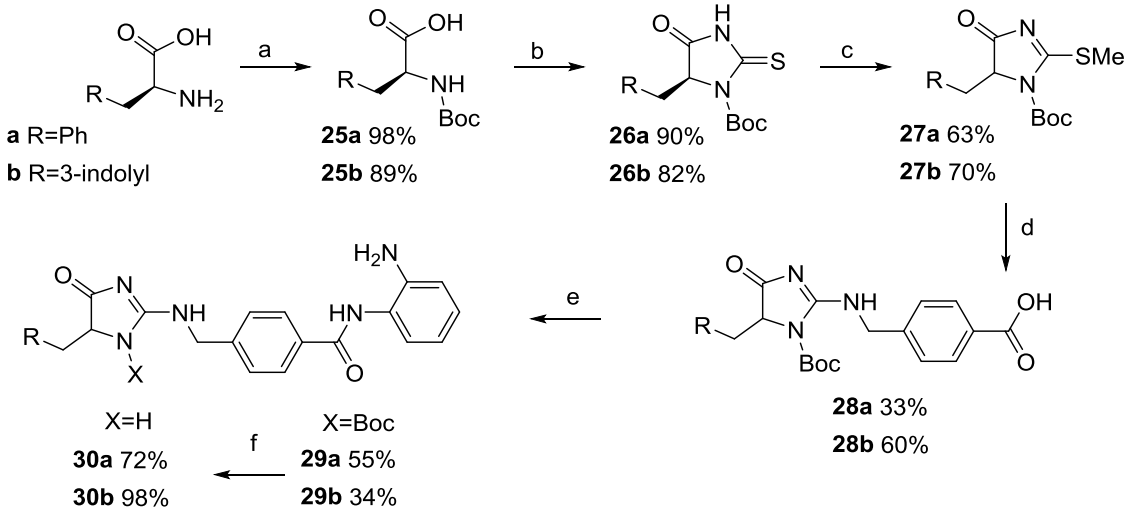
Yiannaki had attempted to remove the acetyl protecting group to reveal the desired *N*-H compounds, however her attempts at this were unsuccessful. Briefly; acidic or basic hydrolysis of the final compounds **24a** and **24b** gave decomposition, presumably because of hydrolysis of the benzamide and/or the dihydroimidazole ring. Deacetylation of **(*R*)-21a** was successful, as was the subsequent methylation (despite a mixture of tautomers being observed by NMR), however displacement of methanethiol failed, presumably the acetyl group draws electron density away from the reacting C=N carbon atom, thus increasing the electrophilicity at this centre.

3.1.2 *N*-H dihydroimidazolones

It was anticipated that by synthesising the N(1)-Boc-2-thiohydantoins using an alternative cyclisation procedure,[101] and then following the already developed route (**Scheme 3.1-1**), the Boc-protected dihydroimidazolone inhibitors could be synthesised. It was hoped that the conditions required for Boc-deprotection would be milder than those needed for deacetylation, and therefore deprotection in the final step of the synthesis would be appropriate.

L-Phenylalanine and L-tryptophan were both Boc-protected by standard protocols to give the *N*-Boc amino acids **25a** and **25b** in excellent yield (98% and 89%).[102,103] Reaction of the *N*-Boc amino acids **25a** and **25b** with ethoxycarbonyl isothiocyanate and pyridine furnished the desired *N*(1)-Boc-2-thiohydantoins **26a**/**26b** in good yield (90% and 82%). Selective *S*-methylation gave the *S*-methyl compounds **27a** and **27b** as

expected in 63% and 70% yield. Racemisation occurred at this point in the same way as it had done for the *N*-acetyl compounds, but it also was not discovered until the end of the route. Displacement of methanethiol by 4-(aminomethyl)benzoic acid was initially attempted using the same conditions as for the acetyl-protected compounds (heating at reflux overnight in ethanol). The product no longer crystallised from the cooled reaction mixture as expected, because the Boc group increases the compound's solubility. The desired product **28a** was readily purified by column chromatography, however it was discovered that some Boc-deprotection occurred during the reaction and this is the reason for the low yield (33%). A reduction in the length of reaction to four hours, for **28b,** was accompanied by a near doubling in yield (60%). Amide coupling of the acids with phenylene-1,2-diamine promoted by BOP was again an unclean reaction and the Boc-protected benzamides **29a**/**29b** were obtained in 55% and 34% yield respectively. As anticipated Boc deprotection was facile; stirring with TFA in dichloromethane at room temperature for three hours gave the *N*(1)-H dihydroimidazolone inhibitors **30a** and **30b** in 72% and 98% yield. These compounds exist as a 2:1 mixture of tautomers in DMSO, the minor tautomer has the other endocyclic C=N bond not the exocyclic C=N bond.



Scheme 3.1-2*: a) for* ***25a****: Boc_2O, NaOH, H_2O:THF, rt, 16 h, for* ***25b****: Boc_2O, NEt_3, MeOH, rt, 16 h; b) ethoxycarbonyl isothiocyanate, pyridine, MeCN, rt, 18 h; c) K_2CO_3, iodomethane, MeCN, rt, 2.5 h; d) 4-(methylamino)benzoic acid, EtOH, reflux, 18 h (4 h for* ***27b****); e) BOP, NEt_3, phenylene-1,2-diamine, DMF, rt, 3 h; f) DCM, TFA, rt, 3 h.*

Now that a successful route to synthesise the dihydroimidazolone inhibitors had been developed, the phenylglycine-derived compound was also made, since it more closely resembles the lead compound mocetinostat. However the opposite absolute stereochemistry of amino acid was used because phenylglycine is an unnatural amino acid and the (*R*)-enantiomer was cheaper, this was eventually inconsequential because

racemsation occurred in the methylation reaction anyway.

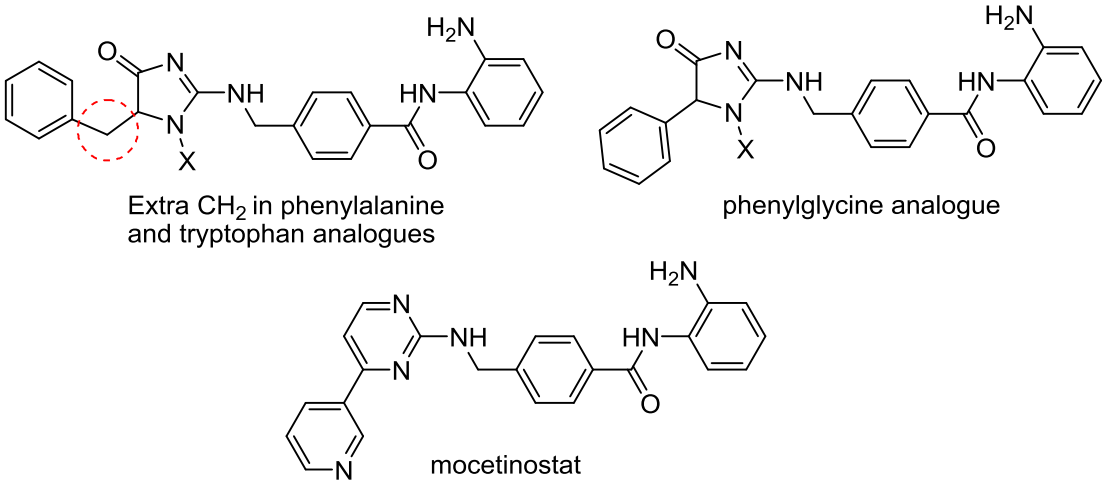
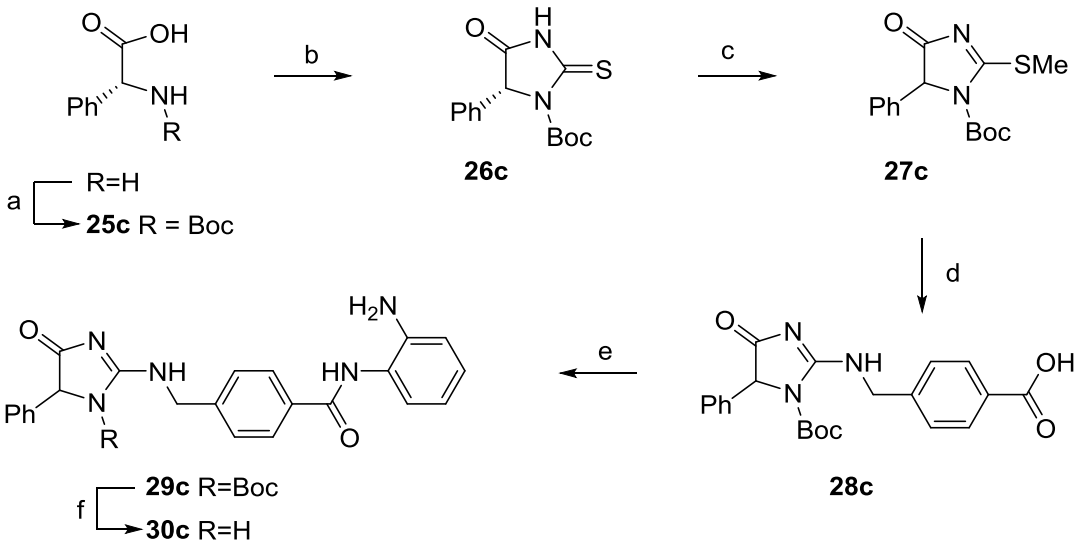


***Figure 3.1-1**: Extra CH_2 present in phenylalanine and tryptophan analogues*

Therefore the phenylglycine-derived analogue **30c** was synthesised according to the same route; phenylglycine was reacted with Boc_2O to give **25c**, which was then cyclised with ethoxycarbonyl isothiocyanate to give the 1-Boc-protected 2-thiohydantoin **26c** in 56% yield (2 steps). *S*-methylation with methyl iodide proceeded in near quantitative yield; however the subsequent displacement with 4-(aminomethyl)benzoic acid gave the acid **28c** in only 39% yield. BOP-mediated amide coupling with phenylene-1,2-diamine proceeded in an unexpectedly good yield, giving **29c** in 98% yield. The Boc group was removed using TFA in dichloromethane to give the phenylglycine analogue **30c** in 70% yield.



***Scheme 3.1-3**: a) Boc_2O, $NaHCO_3$, $THF:H_2O$, rt, 17 h, 95%; b) ethoxycarbonyl isothiocyanate, pyridine, MeCN, rt, 18 h, 59%; c) iodomethane, K_2CO_3, MeCN, rt, 2.5 h, 99%; d) 4-(aminomethyl)benzoic acid, EtOH, reflux, 4 h; 39%; e) phenylene-1,2-diamine, BOP, NEt_3, DMF, rt, 4 h, 98%; f) TFA, DCM, rt, 3 h, 70%.*

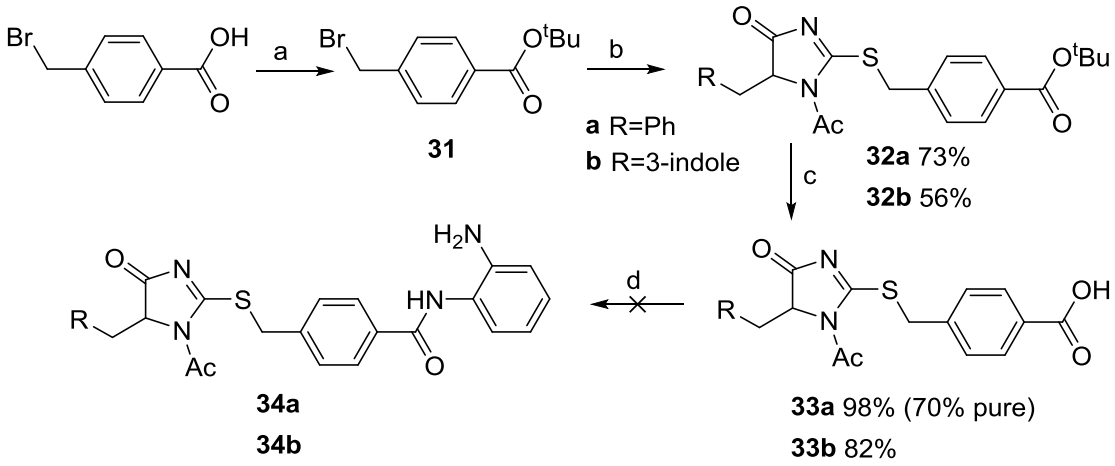
3.1.3 Thioether-linked dihydroimidazolones

A previous attempt to synthesise a thioether-linked dihydroimidazolone by Yiannaki had started with a successful alkylation of thiohydantoin **(*S*)-21a** using methyl-(4-bromomethyl)benzoate (80% yield). However the basic conditions used to attempt the methyl ester hydrolysis failed, giving decomposition products.

It was decided to change to using an acid-labile protecting group for the carboxylic acid. It was hoped that hydrolysis of the *tert*-butyl ester would be possible without causing degradation of the product. Therefore 4-(bromomethyl)benzoic acid was heated at reflux in thionyl chloride for 3.5 hours; the resulting acid chloride was then dissolved in anhydrous diethyl ether and potassium *tert*-butoxide was added portionwise over 1.5 hours to give *tert*-butyl ester **31** (**Scheme 3.1-4**). Thiohydantoins **(*S*)-21a** and **(*S*)-21b** were then readily alkylated with **31** in moderate yield (73% and 56% respectively).

Deprotection of *tert*-butyl ester **32a** was attempted using a 1:1 ratio of TFA in dichloromethane, at room temperature for two hours. The crude product **33a** was isolated in 98% yield but there was a substantial amount of inseparable impurity (30% by ^{1}H NMR). Reducing the concentration of TFA to 33% and reducing the reaction time to 45 minutes for **32b** gave pure **33b**, which was isolated in 82% yield. However, subsequent BOP-mediated coupling of **33b** with phenylene-1,2-diamine failed. TLC indicated that **33b** had been fully consumed but the desired product **34b** was not isolated after column chromatography.

It was hoped that by reacting the impure sample of **33a** with phenylene-1,2-diamine the desired product might be separable from the impurity. However the crude product from the coupling reaction was a complex mixture and column chromatography failed to give any of the desired product **34a**. One isolated side product was identified by ^{1}H NMR as **35** (**Figure 3.1-2**), which presumably was formed as a result of being displaced from the dihydroimidazol-4-one ring, possibly by hydroxybenzotriazole, possibly suggesting that the desired product might not be very stable.



***Scheme 3.1-4**: a) $SOCl_2$, reflux, 3.5 h, then Et_2O and t-BuOK, rt 1.5 h. b) **(S)-21a** or **(S)-21b**, NEt_3, MeCN, 40 °C, (for **(S)-21a: 1**5.5 h) (for **(S)-21b:** 2.5 h). c) for **32a:** TFA:DCM 1:1, rt, 2 h OR for **32b** TFA:DCM 1:2, rt, 45 min. d) BOP, NEt_3, phenylene-1,2-diamine, DMF, rt, 3.5 h.*

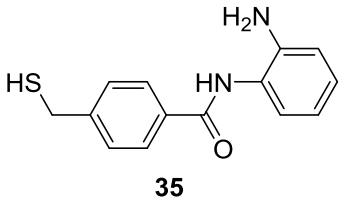


***Figure 3.1-2**: Side product **35**.*

Owing to the difficulties encountered above whilst synthesising the thioether analogues it was envisaged that a convergent synthetic approach may be more successful. If 4-(bromomethyl)benzoic acid could be coupled with phenylene-1,2-diamine prior to alkylation of the 2-thiohydantoin, then no protecting group for the carboxylic acid would be required. This would provide a highly expedient route to the thioether analogues, as it is expected that alkylation with **36** would proceed smoothly.

Several conditions were attempted for the coupling (**Figure 3.1-3**) however all were unsuccessful (Table 3.1-1); evidently the benzylic bromide was too reactive, leading to a mixture of side products. From one such attempted coupling using BOP as the coupling reagent, a side product was isolated that was identified by ^{1}H NMR as **37**, the coupled product but containing a hydroxybenzotriazole unit in place of the bromide**.** Hydroxybenzotriazole is an excellent alpha-effect nucleophile, and unsurprisingly it had reacted with the very electrophilic benzylic bromide. Hydroxybenzotriazole is released from BOP during the coupling process and it acts as a nucleophilic catalyst after its release; since it is not only an excellent nucleophile but a good leaving group also. It was therefore hoped that the isolated product **37** could be used to alkylate the 2-

thiohydantoins directly; however after heating thiohydantoin **(*S*)-21a**, hydroxybenzotriazole side product **37** and NEt_3 at 60 °C for three hours no reaction was observed by ^1H NMR.

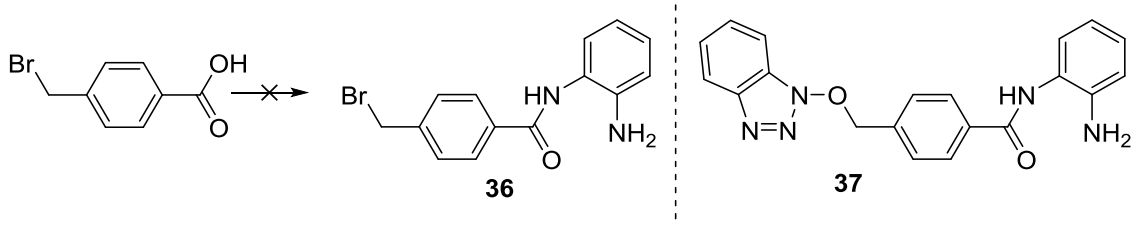
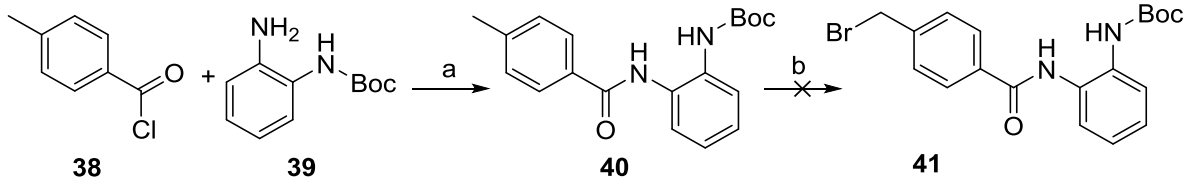


***Figure 3.1-3**: Attempted synthesis of **36** (Table 3.1-1 for conditions). **37**, an isolated side product*

BOP, NEt_3, phenylene-1,2-diamine, DMF, rt, 4 h.
EDC, phenylene-1,2-diamine, DCM, rt 8 h.
a) oxalyl chloride, DMF, DCM, 0 °C, 3 h; b) phenylene-1,2-diamine, toluene:hexane, rt, 16 h.
a) $SOCl_2$, reflux, 3.5 h ; b) phenylene-1,2-diamine, pyridine, Et_2O, rt, 30 min.

Table 3.1-1: Failed conditions attempted for the above coupling

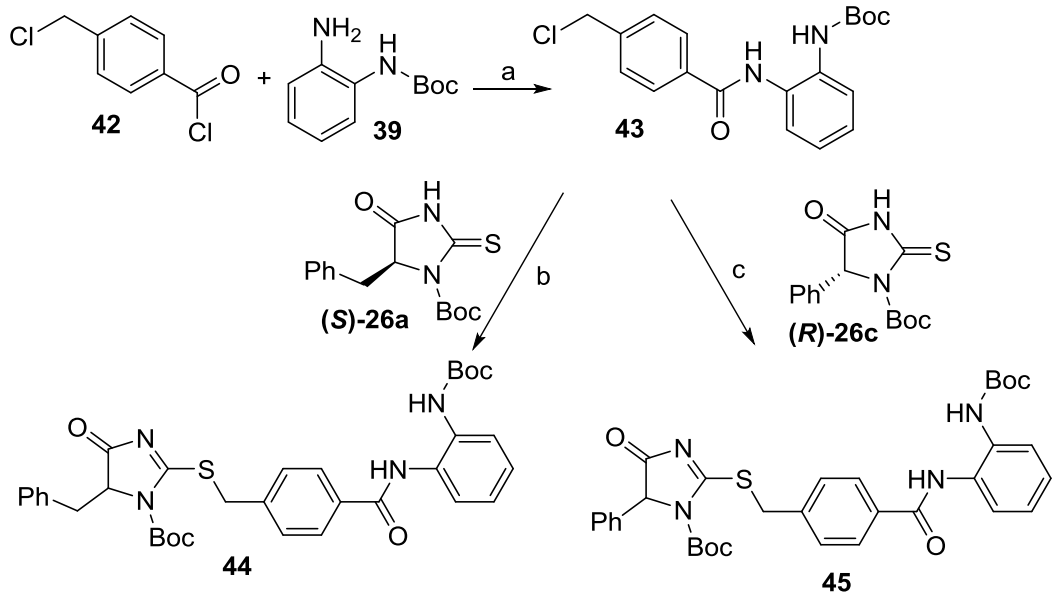
An alternative convergent route was attempted by forming the amide bond before installing the benzyl bromide via a benzylic bromination reaction (Scheme 3.1-5). The reaction of *p*-toluoyl chloride **38** with Boc-protected phenylene-1,2-diamine **39** proceeded smoothly giving the benzamide **40** in 91% yield. A survey of the literature indicated that it might be possible to carry out the selective "side-chain" bromination whilst avoiding aromatic bromination,[104] therefore benzamide **40** was heated at reflux with *N*-bromosuccinimide (NBS) and 10 mol% benzoyl peroxide in carbon tetrachloride for 5.5 h. After this time the starting material had been completely consumed and there were 2 overlapping new spots by TLC. ^1H NMR of the crude product indicated that none of the desired side-chain bromination had occurred but that aromatic bromination had taken place instead.



Scheme 3.1-5: a) NEt_3, DCM, rt, 4.5 h, 85%; b) NBS, benzoyl peroxide, CCl_4, reflux, 5.5 h, 0%.

If the benzyl chloride could be prepared then a Finkelstein reaction could be used to

generate the benzyl iodide *in situ* as Moradei *et al.* had done previously.[105] Therefore the required 4-chloromethyl benzamide **43** was synthesised from 4-chloromethyl benzoyl chloride **42** and Boc-protected phenylene-1,2-diamine **39**. Reaction of this benzyl chloride **43** with sodium iodide, potassium carbonate and a Boc-protected thiohydantoin gave the corresponding phenyl and benzyl substituted thioether analogues **44** and **45** in low yield, 28% and 30% respectively. The low yield reflects the formation of side products as opposed to incomplete reactions. One major side product (15-20% yield) was identified by ^{1}H and ^{13}C NMR as the product that had arisen from alkylation of the carbon atom of the thiohydantoins. TFA deprotection of the bis-Boc-protected compounds was not a clean reaction in either case; TLC indicated the formation of many products. Column chromatography enabled isolation of the products but only at a purity of ~85-90%, because impurities co-eluted from the column. The small amount of each product isolated meant that recrystallization was not attempted and no further efforts were made to synthesise the thioether-linked dihydroimidazolones.



***Scheme 3.1-6**: a) NEt_3, DCM, -10 °C – rt, 1 h, 91%; b) i) NaI, acetone, reflux, 2 h, ii) K_2CO_3, DCM, rt, 20 h, 28%; c) K_2CO_3, NaI, acetone, DCM, reflux, 25 h, 30%.*

3.2 Synthesis of reduced heterocycles

Owing to racemisation of the dihydroimidazolone ring system and the low inhibitory activity of those compounds in cells (see section 3.3.3), a new series of compounds were targeted. It was anticipated that the cellular inactivity could have been caused by the highly polar nature of the heterocycle, giving the compounds poor membrane permeability. Heterocycles which didn't contain a carbonyl group on the ring would be less polar (

Figure **3.2-1**), and furthermore the potential for racemisation would be greatly reduced in such ring systems. Accordingly further series of analogues without the carbonyl group on the heterocycle were synthesised.

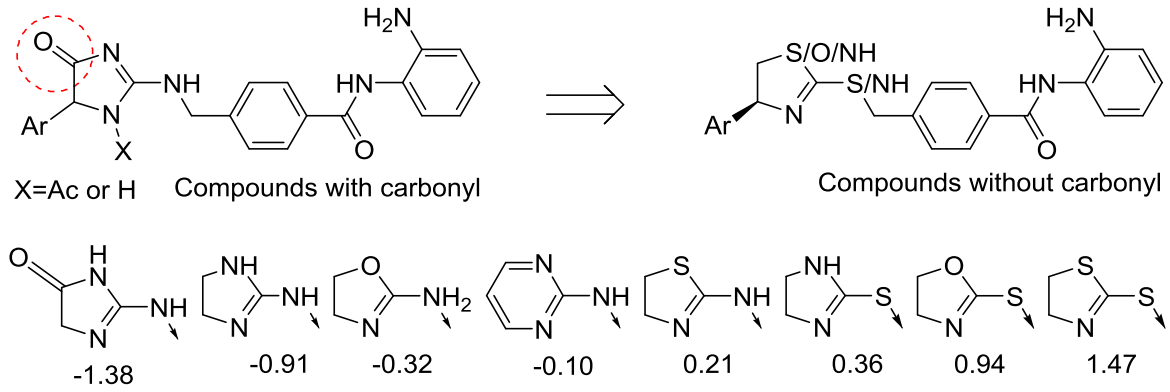
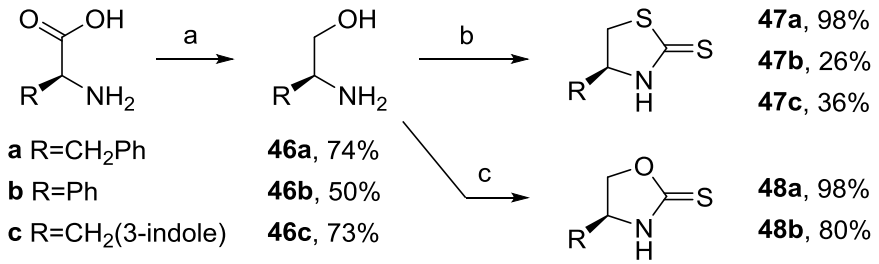


***Figure 3.2-1**: cLogP of heterocycle fragments as calculated by MarvinSketch 6.3.*

3.2.1 Thioether-linked dihydrothiazoles and dihydrooxazoles

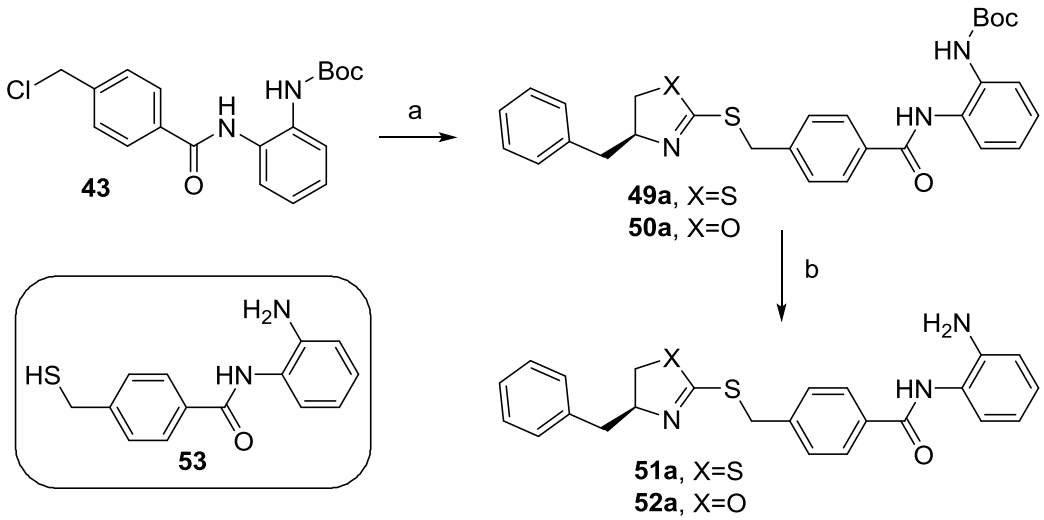
Reduction of L-phenylalanine with borane produced *in situ* from the reaction of sodium borohydride and iodine, gave L-phenylalaninol **46a** in 74% yield. This amino alcohol was then reacted with carbon disulfide to give either the thiazolidine-2-thione **47a** or the oxazolidine-2-thione **48a,** depending on the conditions, in 98% yield. The same procedures were applied to L-phenylglycine to make the phenyl substituted thiazolidine-2-thione **47b** and oxazolidine-2-thione **48b**, and to L-tryptophan for the thiazolidine-2-thione **47c** (**Scheme 3.2-1**).



***Scheme 3.2-1**: a) $NaBH_4$, I_2, THF, reflux, 16-18 h; b) CS_2, KOH, H_2O, reflux, 16 h; c) CS_2, K_2CO_3, EtOH, 50 °C then H_2O_2, 5 min.*

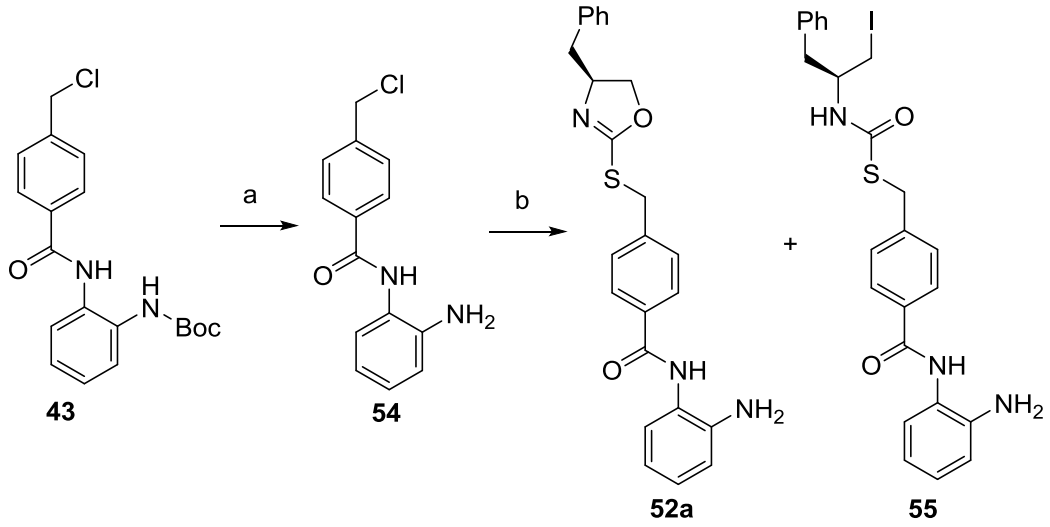
The convergent approach developed above (**Scheme 3.1-6**) was applied; *S*-alkylation of both thiazolidine-2-thione **47a** and oxazolidine-2-thione **48a** was readily achieved in moderate yield (61% and 59% respectively) using the preassembled 4-chloromethylbenzamide **43** and *in situ* Finkelstein conditions.[105] Removal of the Boc-protecting group using TFA was successful in the case of the dihydrothiazole **49a** although it was not a clean reaction and gave moderate yield (56%). However the

attempt to remove the Boc-protecting group from dihydrooxazole **50a** was unsuccessful; none of the desired product **52a** was isolated, instead thiol **53** was isolated in 50% yield.



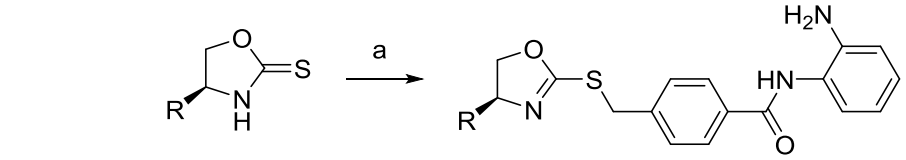
***Scheme 3.2-2**: a) i) NaI, acetone, reflux, 2 h, ii) K_2CO_3, **47a** or **48a**, DCM, rt, 23 h, 61% for **47a** and 59% for **48a**, b) TFA, DCM, rt, 2.5 h, 56% for **49a** and 0% for **50a**.*

It was thought that perhaps the dihydrooxazole ring was not stable to the conditions required for Boc removal. Since the Boc-protecting group in alkylating agent **43** is not required for the alkylation reaction (the aryl amine is not very nucleophilic, owing to the delocalisation of its lone pair of electrons) removal of the Boc group prior to alkylation should provide an alternative route. Therefore benzyl chloride **43** was deprotected using TFA in dichloromethane to give the alkylating agent **54** in 73% yield. However the attempt to alkylate **47a** with **54** using the Finkelstein conditions gave several spots by TLC; although the product **52a** was isolated it was only 90% pure and obtained in low yield (30%). One minor side product (isolated in 7% yield) was identified by ^{1}H NMR as the iodide ring opened product **55**. Iodide's high nucleophilicity, and formation of the strong C=O bond both contribute to the formation of this side product.



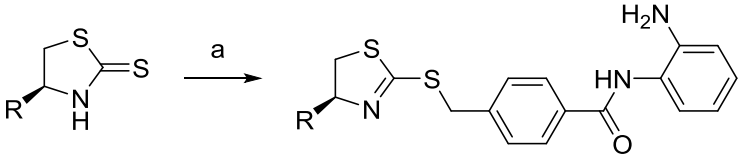
***Scheme 3.2-3**: a) TFA, DCM, rt, 2.5 h, 73%; b) **48a**, K_2CO_3, NaI, acetone, reflux, 2.5 h, 30% **52a** (90% pure) and 7% **55**.*

To overcome the problem of the dihydrooxazole ring being opened by iodide, the alkylation reaction was repeated without the sodium iodide, since the benzyl chloride itself could be an effective electrophile for the alkylation. This was the case; stirring **54** with **48a** and potassium carbonate in acetone at reflux for 16.5 h gave the desired product **52a** in 70% yield after purification. This procedure was also effective for alkylation of the phenyl substituted oxazolidine-2-thione **48b** (Scheme 3.2-4) and the thiazoline-2-thiones **47b** and **47c** (**Scheme 3.2-5**).



a R=CH_2Ph **48a** **52a**, 70%
b R=Ph **48b** **52b**, 47%

*Scheme 3.2-4: a) **54**, K_2CO_3, acetone, reflux, 16-18 h.*



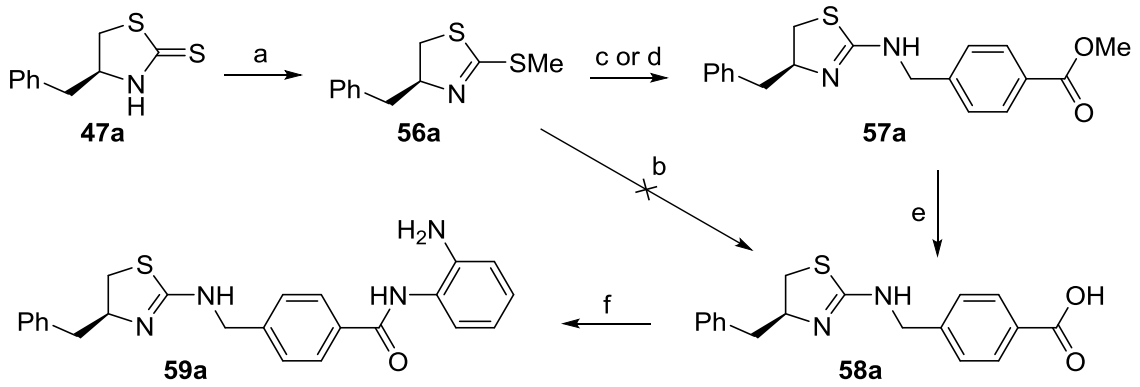
b R=Ph **47b** **51b**, 62%
c R=CH_2(3-indole) **47c** **51c**, 86%

***Scheme 3.2-5**: a) **54**, K_2CO_3, acetone, reflux, 16-18 h.*

3.2.2 Amino-linked dihydrothiazoles and dihydrooxazoles

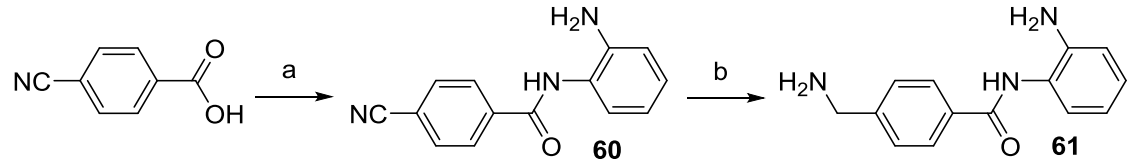
By analogy with **Scheme 3.1-1** and Scheme 3.1-2 the preparation of **59a** was attempted using a reaction in which methanethiol is displaced from the heterocycle

(**Scheme 3.2-6**). First thiazolidine-2-thione **47a** was readily methylated in quantitative yield following a literature procedure for methylation of the unsubstituted dihydrothiazolidine-2-thione.[106] Heating **47a** at reflux with iodomethane in acetone formed the hydroiodide salt from which the free base **56a** was liberated using $NaHCO_3$. Displacement of methanethiol by 4-(aminomethyl)benzoic acid failed; only starting material was recovered. 4-(aminomethyl)benzoic acid exists as a zwitterion and it is likely that the presence of the carboxylic acid group rendered the amine unreactive in this reaction. This displacement reaction did work well with the dihydroimidazolone compounds (**Scheme 3.1-1**, Scheme 3.1-2 and Scheme 3.1-3), however the carbonyl group on the heterocycle may increase the electrophilicity of the reacting carbon atom, thus activating the system. It was hoped that methyl 4-(aminomethyl)benzoate would displace methanethiol from **56a** then the methyl ester could be hydrolysed to reveal the carboxylic acid. Because the hydrochloride salt of methyl-4-(aminomethyl)benzoate was available it was heated at reflux with dihydrothiazole **56a** and potassium carbonate in ethanol; however, no reaction had occurred after 48 h and starting materials were recovered. It was believed that the heterogeneous base used may not have successfully deprotonated the hydrochloride salt of methyl-4-(aminomethyl)benzoate since the starting materials did not dissolve. The reaction was repeated using DIPEA in place of potassium carbonate and this gave the desired product **57a** in 63% yield, after 24 h at reflux. Hydrolysis of the resulting methyl ester was achieved in 61% yield using lithium hydroxide in THF and water. BOP-mediated amide coupling of the acid **58a** and phenylene-1,2-diamine was successful, but the reaction mixture contained many spots by TLC. Purification by column chromatography twice and trituration with hot diethyl ether gave a very low yield (7%) of the desired benzamide **59a** (a significant amount of product was lost during purification).



***Scheme 3.2-6**: a) iodomethane, acetone, reflux, 3 h, then $NaHCO_3$, 99%; b) 4-(aminomethyl)benzoic acid, EtOH, reflux, 48 h; c) methyl 4-(aminomethyl)benzoate.HCl, K_2CO_3, EtOH, reflux, 48 h, 0%; d) methyl 4-(aminomethyl)benzoate.HCl, DIPEA, EtOH, reflux, 24 h, 63%; e) LiOH, H_2O:THF, rt, 26 h, 61%; f) phenylene-1,2-diamine, BOP, NEt_3, DMF, rt, 4 h, 7%.*

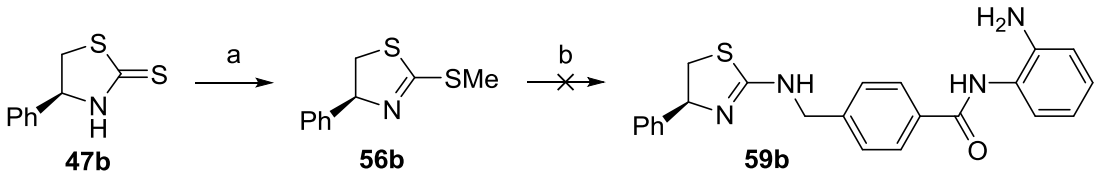
A more convergent approach was sought because previously the amide couplings with phenylene-1,2-diamine had often been low yielding and purification of the products had been difficult. For this reason preassembled benzamide linker **61** was synthesised according to the procedure of Xian-Ping *et al.* (**Scheme 3.2-7**).[107] It was expected that this amine **61** could be used instead of using methyl 4-(aminomethyl)benzoate in the displacement reaction that releases methanethiol.



***Scheme 3.2-7**: a) EDC, NEt_3, HOBt, phenylene-1,2-diamine, DMF, rt, 20 h, 56%; b) H_2, 10% Pd/C, H_2SO_4, MeOH, rt, 5.5 h, 62%.*

So dihydrothiazole **56b** was stirred with **61** in ethanol at reflux for 24 h, but after this time no reaction had occurred, so heating was continued for a further 6 days after which time a small amount starting materials still remained. Purification of the crude product was attempted by column chromatography but the desired product **(*S*)-59b** was not isolated because of the complex mixture of products. Repeating the reaction at a higher temperature (120 °C) in *n*-butanol for 5 h gave the same result. The hydroiodide salt of **56b** was reacted with **61** in ethanol at reflux overnight but this also gave a complicated mixture of products and starting materials. A neat melt of the two solids **56b** and **61** was also attempted at 180 °C for 1 h. This again gave a complicated mixture of products and starting materials, interestingly some liquid condensed around the neck of the flask in the melt reaction, which is presumed to be water formed from the

dehydration of the *N*-(2-aminophenyl)benzamide giving either **63** or **62** (**Figure 3.2-2**).



Scheme 3.2-8*: a) iodomethane, acetone, reflux, 3 h, then $NaHCO_3$, 88%; b)* ***61****, ethanol, reflux, 7 days; OR* ***61****, n-butanol, reflux, 5 h; OR hydroiodide salt of* ***56b****,* ***61****, ethanol, reflux, 17 h; OR* ***61****, 180 °C (melt), 1 h.*

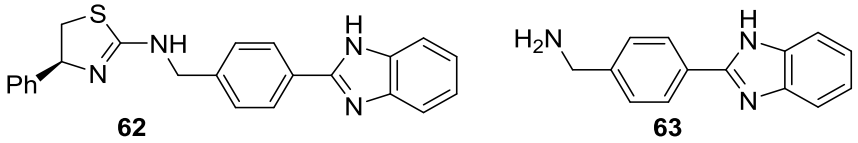
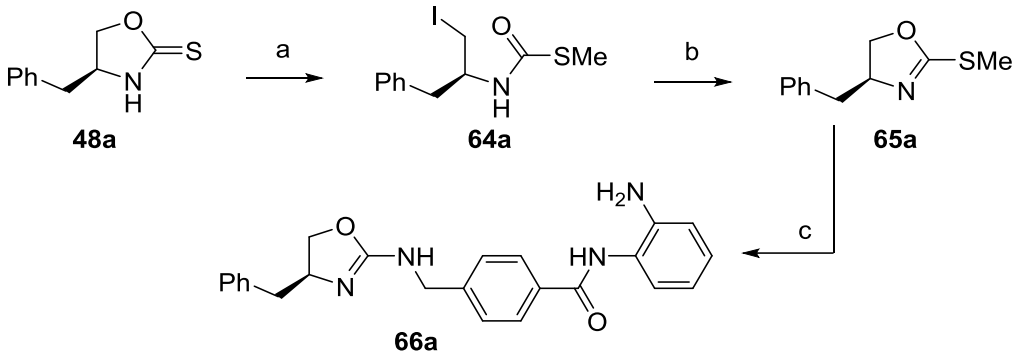


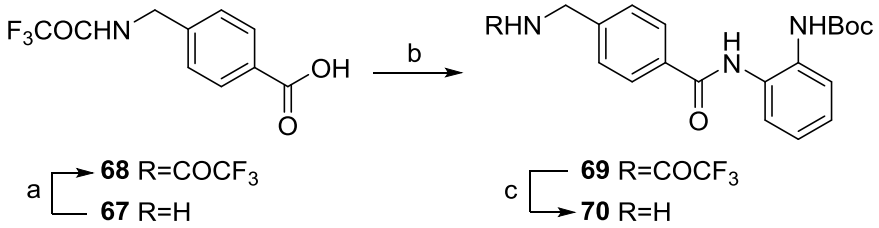
Figure 3.2-2*: Possible benzimidazole side products from the dehydration of N-(2-aminophenyl)benzamides.*

Treating oxazolidine-2-thione **48a** to the conditions used for methylation of thiazoline-2-thione **47a** (**Scheme 3.2-6**) gave iodide **64a**, which presumably arose from iodide ring opening of the desired product **65a** in a similar manner to the formation of **55** (**Scheme 3.2-3**). It had been expected that the dihydrooxazole ring would be inherently less stable than its thiazole equivalent, because of the stronger C=O bond formed from this ring opening compared to the C=S bond. Iodide **64a** was obtained in high yield (93%) without purification and it was readily converted into the desired 2-methylsulfanyl-4,5-dihydrooxazole **65a** in 90% yield by stirring with potassium carbonate in acetonitrile at reflux. At this point it was anticipated that the previously low-yielding BOP coupling step would be a problem for the dihydrooxazole, as it had been for the dihydrothiazole. It was hoped that it could be avoided by employing a convergent route as attempted, albeit unsuccessfully, for the dihydrothiazole **56b** above. Therefore dihydrooxazole **65a** was heated at reflux with amine **61** for 4.5 hours in ethanol. TLC indicated that the starting materials had been consumed; however after column chromatography only a low yield (26%) of impure product **66a** was isolated, which couldn't be further purified.



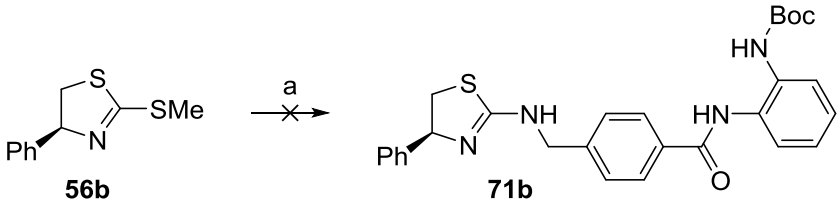
Scheme 3.2-9*: a) iodomethane, acetone, reflux, 2 h, 93%; b) K_2CO_3, MeCN, reflux, 5 h, 90%; c)* ***61****, EtOH, reflux, 4.5 h, 26% (~85% pure).*

It was thought that dehydration of the *N*-(2-aminophenyl)benzamide unit could be causing some of the impurities in the displacement reactions (**Scheme 3.2-8** and **Scheme 3.2-9**) and so the Boc-protected preassembled linker unit **70** was prepared using a different route that was also previously published. First 4-(aminomethyl)benzoic acid was protected according to the solvent-free procedure of Chandra *et al.*[108] as a trifluoroacetate **68** in 85% yield. This was then converted into the acid chloride and coupled to Boc-protected phenylene-1,2-diamine **39**, to give benzamide **69** and finally the trifluoroacetate protecting group was removed with potassium carbonate to give amine **70** (**Scheme 3.2-10**).[109]



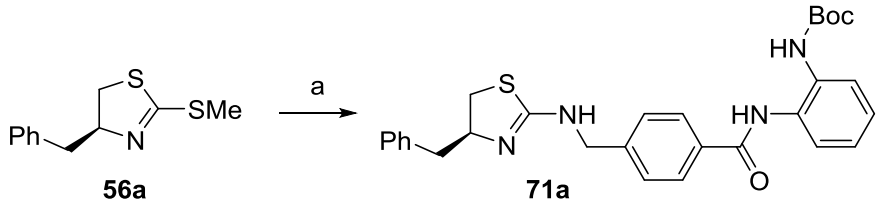
Scheme 3.2-10*: a) trifluoroacetic anhydride, 0 °C to rt, 2 h, 85%; b) Et_3N, $(COCl)_2$, DMF, DCM, 40 °C for 1 h; then* ***39****, pyridine, rt for 20 h, 95%; c) K_2CO_3, H_2O, MeOH, 70 °C, 4 h, 88%.*

Phenyl substituted dihydrothiazole **56b** was stirred with **70** in ethanol at reflux for 48 hours but starting materials remained, switching solvents to *n*-butanol allowed an increase in temperature to 120 °C, but after 48 h the reaction mixture still contained a substantial amount of **56b** and many impurities had formed.



Scheme 3.2-11*: a)* ***70****, ethanol, reflux, 48 h OR n-butanol, reflux, 48 h.*

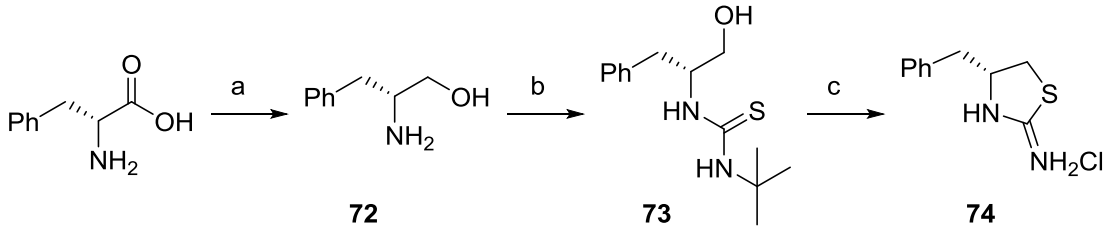
The reaction was also tested with benzyl substituted dihydrothiazole **56a**; stirring at reflux with **70** in ethanol for 48 h gave a small amount of conversion but still **56a** remained. One equivalent of DIPEA was added to help facilitate proton transfers between the intermediates, but after stirring at reflux for 48 h the reaction was still incomplete and impurities were beginning to form. The product **(*S*)-71a** was isolated but only in low yield (19%), and purification was difficult (the product could not be purified to greater than ~80%) so an improved method was sought.



56a **71a**

***Scheme 3.2-12**: a) **70**, ethanol, reflux, 48 h, then DIPEA, reflux, 48 h, 19% (~80% pure).*

Another approach was envisioned in which thiazolidine-2-imine **74** could undergo an alkylation reaction with benzyl chloride **43**. The benzyl substituted thiazolidine-2-imine **74** was prepared using a similar procedure to the work of Carry *et al.;*[110] reaction of amino alcohol **72** with *tert*-butyl isothiocyanate gave the *tert*-butyl thiourea **73** in quantitative yield. Stirring the thiourea with concentrated acid at reflux caused a dehydration cyclisation reaction and *in situ* removal of the *tert*-butyl group to give the imine **74** in 59% yield.

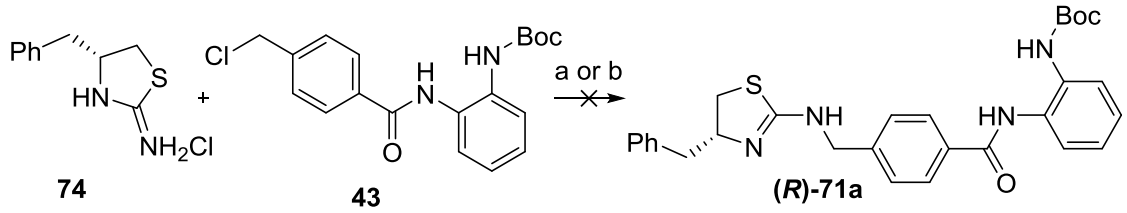


72 **73** **74**

***Scheme 3.2-13**: a) $NaBH_4$, I_2, THF, reflux, 16-18 h, 66%; b)tert-butyl isothiocyanate, ethanol, rt, 16 h, 98%; c) 6M aq. HCl, reflux, 8.5 h, 59%.*

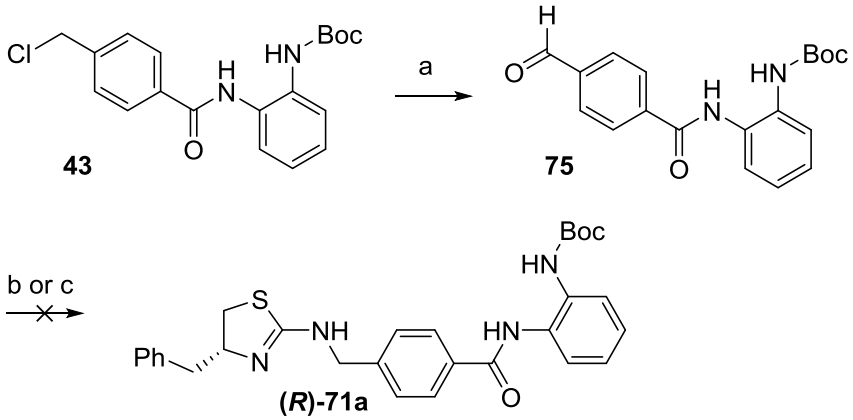
Hirashima *et al.* reported that thiazolidine-2-imines could be alkylated using some benzyl bromides in pyridine,[111] so **74** was stirred with benzyl chloride **43** in pyridine at room temperature, however none of the desired product **(*R*)-71a** was isolated. In another example from the literature Nagarajan *et al.* used an alkyl bromide and potassium carbonate to alkylate thiazolidine-2-imine.[112] This suggested that the benzyl chloride **43** might not be reactive enough to alkylate the imine. Therefore an *in situ* Finkelstein reaction was carried out; imine **74** was stirred with benzyl chloride **43**, potassium bromide and potassium carbonate at reflux. However the reaction gave a

complicated mixture of products and desired product **(*R*)-71a** was not identified in the mixture.



***Scheme 3.2-14**: a) pyridine, rt, 16 h; b) KBr, K_2CO_3, dioxane, reflux, 24 h.*

Another option was a reductive amination reaction with the aldehyde **75**. A similar reductive amination had been found in the patent literature using titanium isopropoxide then sodium borohydride to reductively alkylate a thiazolidine-2-imine with a benzyl ketone.[113] Therefore the benzaldehyde **75** was prepared by a Kornblum oxidation using dimethyl sulfoxide and triethylamine *N*-oxide (**Scheme 3.2-15**-a), a procedure adapted from a patent by Sankyo Company.[114] Subsequently a reductive amination reaction with the free base of **74** was attempted using titanium isopropoxide (**Scheme 3.2-15**-b); however the major product in the reaction was the alcohol **76 (Figure 3.2-3)**. The two part nature of this procedure relies on the methanol added in the second step being completely anhydrous; otherwise the imine intermediate could just be hydrolysed back to the aldehyde by any water in the methanol. Since the desired amination is with an aldehyde which is more reactive than a ketone, the use of titanium isopropoxide might not be necessary. Therefore a standard reductive amination reaction was attempted; the free base of **74** was reacted with aldehyde **75** and sodium triacetoxyborohydride in dichloromethane, but the only product detected was the alcohol **76**.



***Scheme 3.2-15**: a) triethylamine N-oxide, DMSO, DCM, rt, 17 h, 45%; b) **74** (free base), titanium isopropoxide, DCM, rt , 17 h, then anh. MeOH and $NaBH_4$, rt, 2 h; c) **74** (free base), $NaBH(OAc)_3$, DCM, rt 20 h.*

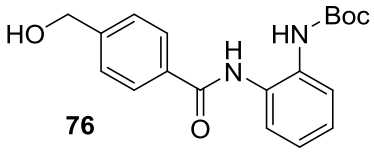
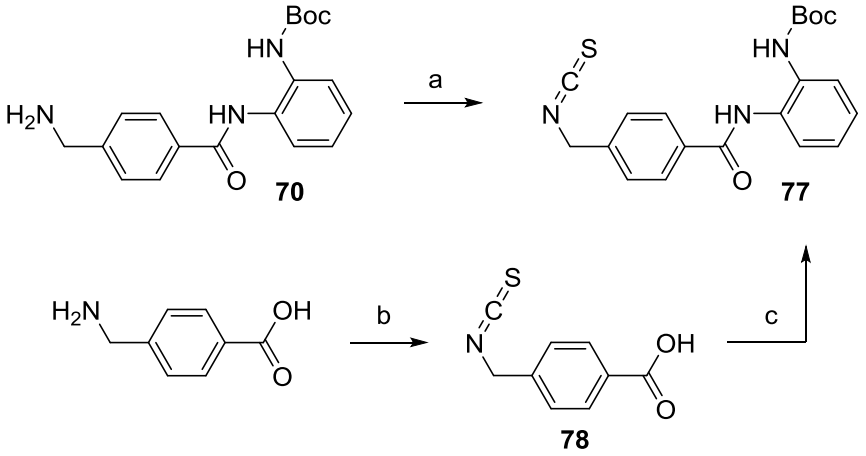


***Figure 3.2-3**: Alcohol side product from the attempted reductive amination.*

To synthesise the amino-linked dihydrothiazoles an alternative approach was necessary. Instead of initially forming the heterocycle and appending the benzamide linker section, the chiral amino alcohol could first be joined to the benzamide linker and then the heterocycle could be formed in the final step. So by analogy to some previous work on 2-aminodihydrothiazoles by Yoshii *et al.*,[115] a benzamide linker bearing an isothiocyanate **77,** could be reacted with amino alcohols to give β-hydroxythioureas, which could undergo cyclisation in concentrated acid in a similar manner to the formation of imine **74** (**Scheme 3.2-13**).

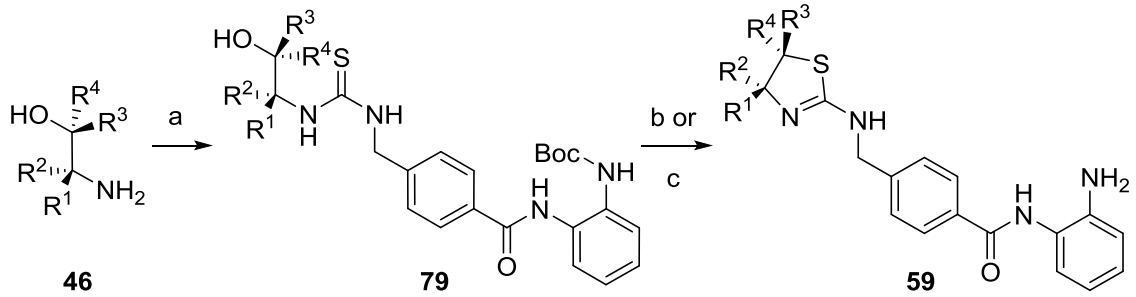
Initially a modified procedure from Nasim *et al.*[116] was used to prepare isothiocyanate **77** from amine **70** which had been made previously (**Scheme 3.2-10**). Reaction of **70** with carbon disulfide, then triethylamine followed by addition of tosyl chloride gave the isothiocyanate **77** in 68% yield (**Scheme 3.2-16**-a). An improved synthesis of **77** directly from 4-(aminomethyl)benzoic acid was subsequently developed. Using methodology from a patent by Nakano and Saito,[117] 4-(aminomethyl)benzoic acid was converted quantitatively into the isothiocyanate **78**, which without purification was then converted into the acid chloride and coupled with Boc-protected phenylene-1,2-diamine **39**, to give **77** in 74% yield (2 steps).



***Scheme 3.2-16**: a) i) DCM, rt, 1 min; ii) NEt_3, rt, 10 min; iii) 0 °C, tosyl chloride, 10 min, 68%; b) i) NEt_3, THF, H_2O, rt, 22 h; ii) 0 °C, I_2, THF, 2 h; c) i) $(COCl)_2$, DCM, DMF, rt then reflux, 2 h, 100%; ii) **39**, DCM, pyridine, 0 °C, 1 h, then rt, 16 h, 74%.*

Reaction of isothiocyanate **77** with chiral amino alcohols **46** gave β-hydroxythioureas **79** in good yield. These thioureas were subsequently ring closed, and

deprotected *in situ*, by stirring at reflux in concentrated hydrochloric acid to give the desired amino-linked dihydrothiazoles **59**. These final products were recrystallized to ensure maximum purity of the final compounds, however a substantial amount of material did not recrystallize and this is part of the reason for the low yield. The dehydration ring closure with concentrated hydrochloric acid failed in the case of **79e**. Consequently, Mitsunobu conditions were applied to **79e** and they afforded the desired thiazoline which was then deprotected to give **59e**.



***Scheme 3.2-17**: a) **77**, MeCN, rt, 16 h; b) Conc. HCl, reflux, 10 min to 75 min (for all except **79e**), c) diethyl azodicarboxylate, Ph_3P, THF, rt, 75 min, then HCl in Et_2O, rt, 5 h (for **79e**). See **Table 3.2-1** below for substituents and yields.*

59	R^1	R^2	R^3	R^4	a) (%)	b) (%)
(*S*)-a	$PhCH_2$	H	H	H	87	10
(*R*)-a	H	$PhCH_2$	H	H	94	22
(*S*)-b	Ph	H	H	H	89	24
(*R*)-b	H	Ph	H	H	96	23
(*R*)-d	H	H	Ph	H	57	21
(*S*)-d	H	H	H	Ph	100	49
e	*p*-HOC_6H_4	H	H	H	67	47
(*R*,*R*)-f	H	Ph	Ph	H	99	36
(*S*,*S*)-f	Ph	H	H	Ph	100	33

***Table 3.2-1**: Substituents and yields for **Scheme 3.2-17**.*

With compounds **79d/f** the stereochemistry of the acidic intramolecular ring closure becomes important. Initially it was thought that the reaction would proceed primarily through an S_N1 type mechanism, since the water leaving group leaves behind a fairly stable benzylic carbocation. However in the case of **79d** a truly S_N1 mechanism would lead to racemisation which was not the case since the products have non-zero optical rotations that are approximately equal and opposite (+38.4 and -35.3 respectively). In order to determine whether **(*R*)-59d** and **(*S*)-59d** had undergone partial racemisation they were sent to GSK and analysed using chiral HPLC by Eric Hortense.

This revealed that partial racemisation had taken place for each compound, **(*R*)-59d** had an enantiomeric excess of 58%, and **(*S*)-59d** had an enantiomeric excess of 46%. This suggests that in these cases the reaction was approximately 50% S_N1 type (stepwise) and 50% S_N2 type (concerted). An S_N1 mechanism might be expected to give a mixture of diastereomers for the two isomers of **59f**, but these were not detected in either case. It is possible that the ~25% minor diastereomer was removed during recrystallization, or there may be diastereocontrol from the adjacent chiral centre. This would be likely to favour formation of *trans*-substituted diastereomer (the same isomer made by an S_N2 type mechanism) arising from ring closure of the least hindered conformation of the carbocation.

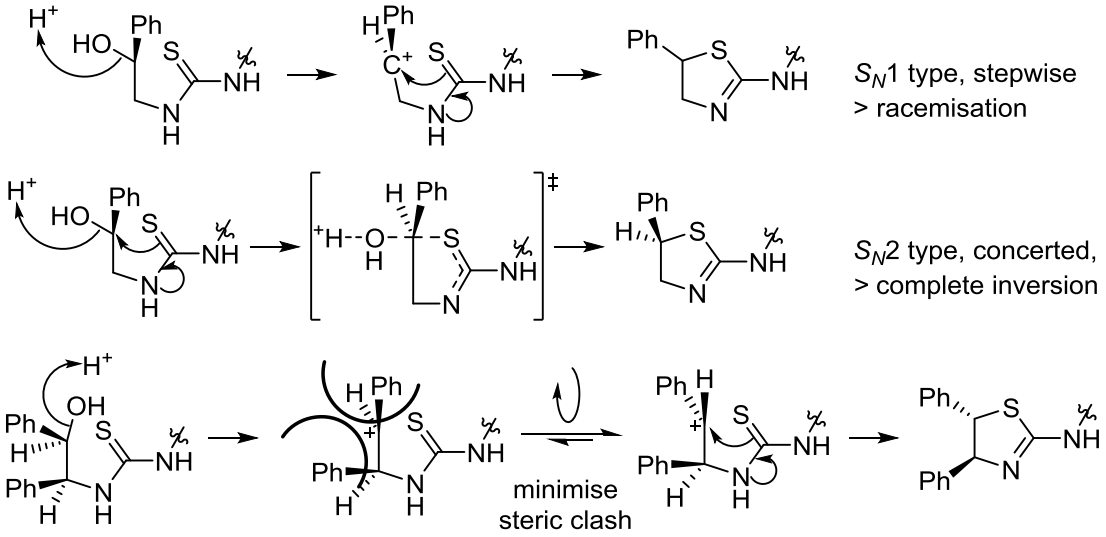
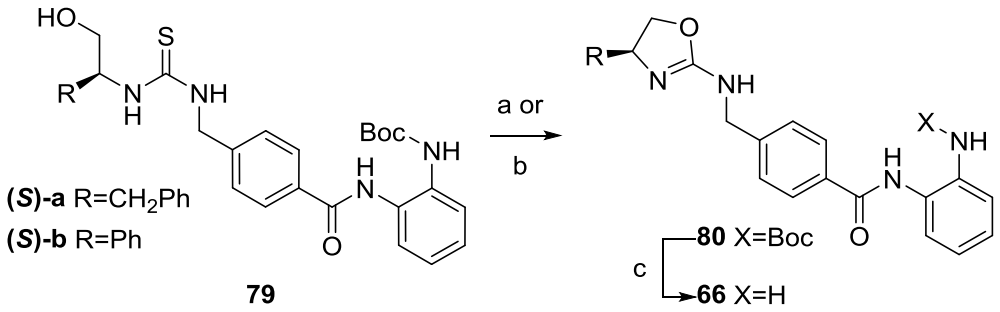


***Figure 3.2-4**: Mechanisms of acidic ring closure and the stereochemistry of products.*

The success of the convergent approach to make amino-linked dihydrothiazoles via β-hydroxythiourea intermediates prompted the application of a similar methodology to make amino-linked dihydrooxazoles from the same β-hydroxythiourea intermediates. A ring closure reaction in which sulfur is the leaving group, instead of oxygen, would afford the desired dihydrooxazole. Yellow mercuric oxide can activate sulfur as a leaving group, and it was used by Uchida *et al.*[118] in an analogous reaction to convert a β-hydroxythiourea into an amino-linked dihydrooxazole. Therefore thiourea **(*S*)-79b** was reacted with yellow mercuric oxide under those conditions (**Scheme 3.2-18**-a), however no conversion of **(*S*)-79b** was detected. Another literature procedure, by different authors, Hirashima *et al.*,[119] used a different solvent mixture and a higher reaction temperature to carry out a similar reaction, so **(*S*)-79b** was subjected to these reaction conditions (**Scheme 3.2-18**-b). After extending the reaction time, and increasing the amount of yellow mercuric oxide added, complete conversion was

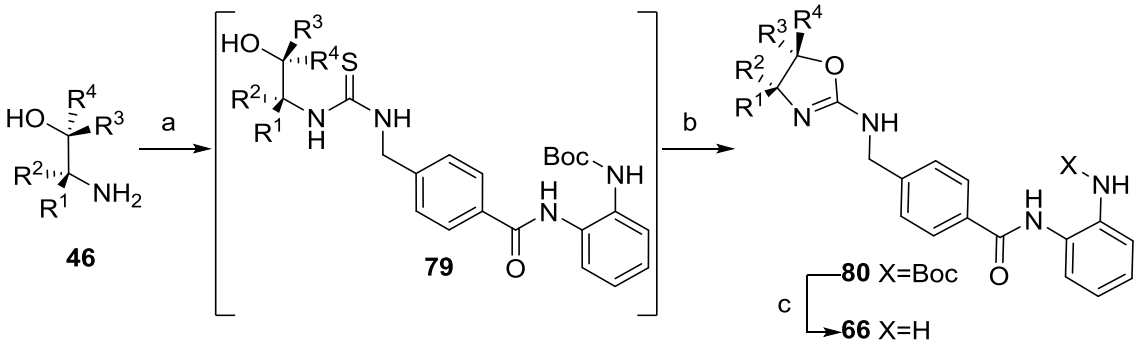
achieved; dihydrooxazole **(*S*)-80b** was isolated in 76% yield. The same reaction with **(*S*)-79a** proceeded very slowly with the same source of yellow mercuric oxide (an old commercial bottle) and after 6.5 h only a small amount of product was detected by TLC. However using freshly prepared yellow mercuric oxide[120] the reaction was complete after 30 min and dihydrooxazole **(*S*)-80a** was isolated in 92% yield. Removal of the Boc-protecting groups from these two dihydrooxazoles using TFA in dichloromethane gave the final compounds **66a** and **(*S*)-66b** in 47% and 74% yield respectively.



***Scheme 3.2-18**: a) (for **(S)-79b**) yellow HgO, 1:2 ethanol:acetone, rt, 22 h, 0%; b) for **(S)-79a:** freshly prepared yellow HgO, 1:2 ethanol:toluene, reflux, 30 min, 92%; for **(S)-79b:** yellow HgO, 1:2 ethanol:toluene, reflux, 10 h, 76%; c) TFA, DCM, rt, 4 h, 47% for **(S)-80a**, 74% for **(S)-80b**.*

Mercury and its salts are very toxic, so preparing and using yellow mercuric oxide are hazardous procedures, for this reason an alternative, safer method of forming the dihydrooxazole ring was sought. Another method of activating the sulfur as a leaving group is to exploit its nucleophilicity by making the methyl thioether so that methanethiol (a good, volatile leaving group) can be lost upon formation of the dihydrooxazole, as in **Scheme 3.1-1** and **Scheme 3.1-2**. This intra molecular approach has been used once before by Li *et al.* in 2011 to make a series of amino-linked dihydrooxazoles from amino alcohols and an isothiocyanate.[121] They only reported the reaction using cyclic amino alcohols, and the thiourea intermediates were not isolated. Following their procedure, amino alcohol **(*R*)-46b** was stirred with isothiocyanate **77** in THF overnight, after this time the THF was evaporated, and then the residue was dissolved in acetone and stirred with iodomethane for 9 h before being quenched with sodium hydrogen carbonate (**Scheme 3.2-19**). The crude product was less pure than that from the reaction using yellow mercuric oxide, but after easy purification the dihydrooxazole **(*R*)-80b** was obtained in 41% yield. This procedure was then applied to a selection of amino alcohols that were either commercially available, or were made by reduction of the corresponding amino acid with sodium borohydride and iodine. When purification of the amino alcohol was difficult it was not purified before it was reacted

with isothiocyanate **77**, but in those cases the β-hydroxythiourea intermediate was purified. Removal of the Boc-protecting group using TFA in dichloromethane gave the desired final compounds in moderate to good yield.



Scheme 3.2-19*: a)* ***77****, THF, rt, 16 h; b) iodomethane, acetone, rt, 4-9 h; c) TFA, DCM, rt, 4 h.*

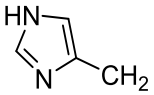
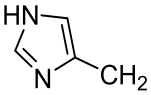
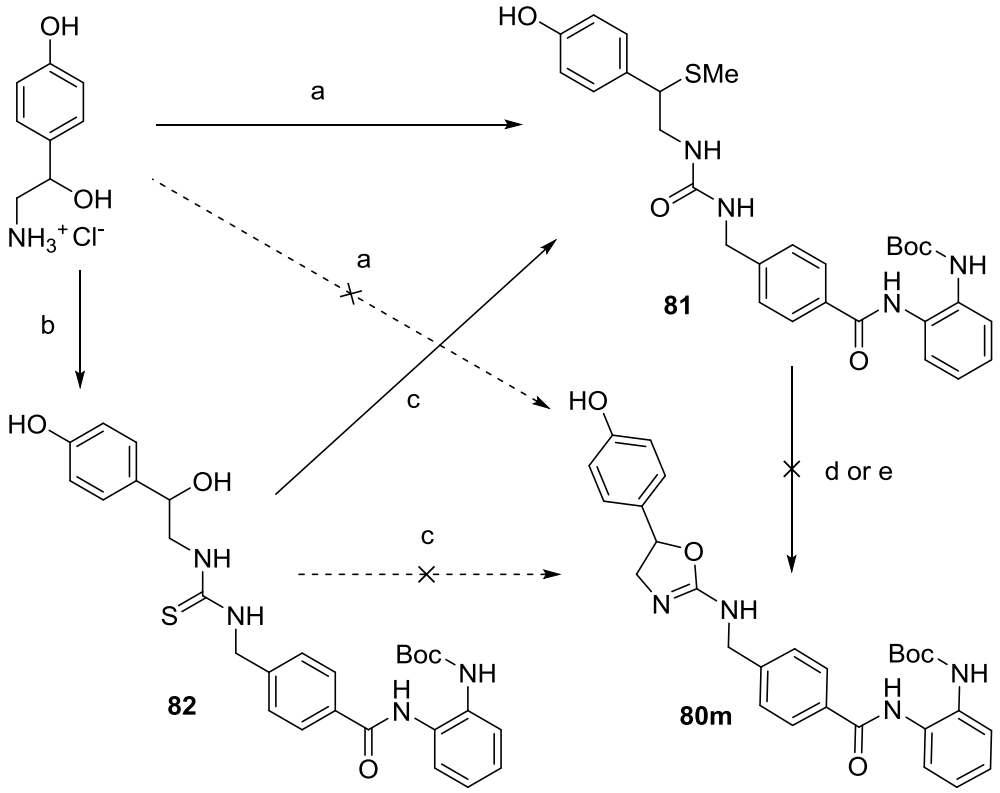
66	R^1	R^2	R^3	R^4	a) & b) (%)	c) (%)
(*R*)-b	H	Ph	H	H	41	32
(*S*)-d	H	H	Ph	H	71	34
(*R*)-d	H	H	H	Ph	75	34
e	*p*-HOC_6H_4	H	H	H	67	70
(*R*,*S*)-f	H	Ph	Ph	H	85	31
(*S*,*S*)-f	Ph	H	Ph	H	56	98
g	Ph	Ph	H	H	99	59
(*S*)-h		H	H	H	33	22
(*R*)-h	H		H	H	43	54
i	(±) *p*-FC_6H_4 / H		H	H	67	61
j	(±) *m*-FC_6H_4 / H		H	H	76	86
k	(±) *p*-$F_3CC_6H_4$ / H		H	H	69	71
m	H	H	(±) *p*-HOC_6H_4		0	-

Table 3.2-2*: Substituents and yields for* ***Scheme 3.2-19****.*

This strategy was unsuccessful in the synthesis of the 4-hydroxyphenyl compound **66m** derived from octopamine (**Scheme 3.2-20**), instead the methylsulfanyl substituted urea **81** was isolated. During the reaction thiourea **82** was not isolated, and because the urea **81** has a very similar R_f to thiourea **82** the reaction was left for longer than usual because it appeared by TLC that no reaction was taking place. The reaction

was repeated and the thiourea **82** intermediate was purified, then the ring closure step was carried out in an open flask (as opposed to under a nitrogen balloon) to encourage the methanethiol to evaporate. After only 4 h TLC showed that the urea **81** was the major product. Reverting to the use of freshly prepared yellow mercuric oxide also did not afford the desired dihydrooxazole **80m**. It was envisioned that **81** might undergo an intramolecular cyclisation at elevated temperature, and so it was stirred at reflux in propan-2-ol (83 °C) for 48 h but no reaction was observed by TLC.



Scheme 3.2-20*: a)* ***77****, K_2CO_3, THF, reflux, 90 h, then: iodomethane, acetone, rt, 40 h, 30%; b)* ***77****,K_2CO_3, THF, reflux, 96 h, 94%; c) iodomethane, acetone, rt, 4 h; d) freshly prepared yellow HgO, 1:2 ethanol:toluene, reflux, 6.5 h; e) ^{i}PrOH, reflux, 48 h.*

The electron rich aromatic ring presumably provided an electronic ‘push’ to open the dihydrooxazole, once it had been made and protonated by the hydrogen iodide formed in the reaction, to give the stable urea group. The oxonium species **83** must then have then been trapped by methanethiol that was dissolved in the solvent (acetone).

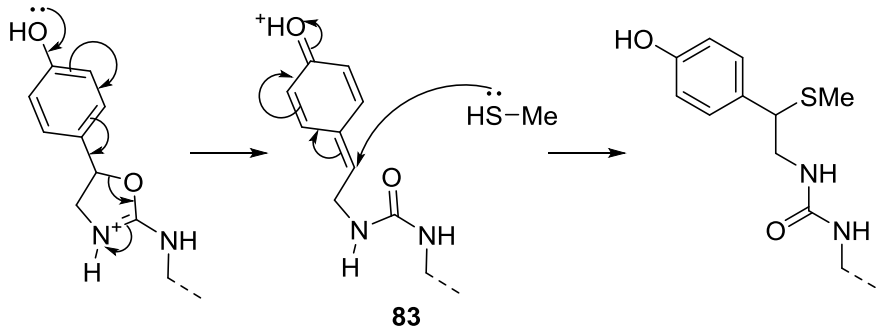
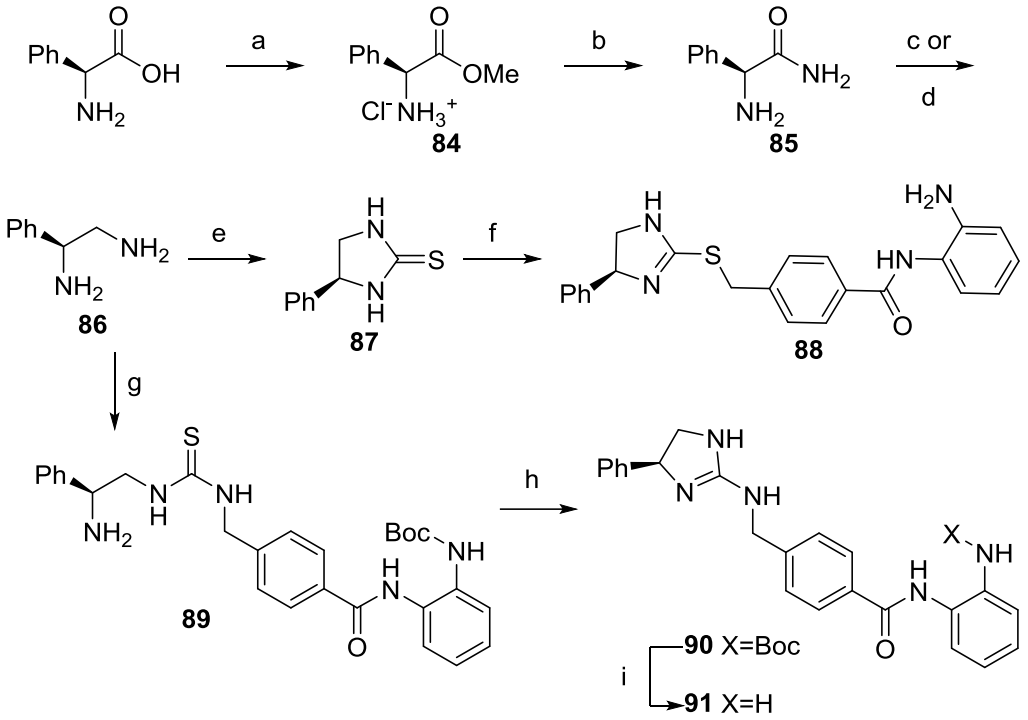


Figure 3.2-5*: Proposed mechanism for the formation of* ***81****.*

3.2.3 Dihydroimidazoles

Synthesis of chiral amino- and thioether-linked dihydroimidazole rings via the above convergent routes required access to chiral 1,2-diamines which are not commercially available. Based on the enzyme assay results of the compounds previously made (see **Table 3.3-1** and **Table 3.3-2**) it was decided to make the phenyl substituted dihydroimidazoles only. The required diamine, (*S*)-1-phenylethane-1,2-diamine **86** was prepared by either a lithium aluminium hydride, or sodium borohydride and iodine, reduction of (*S*)-phenylglycine amide **85.** Both of these procedures afforded impure products that could not be easily purified, however purification after reaction with either carbon disulfide or isothiocyanate **77** was successful giving the imidazolindine-2-thione **87** (31%) or β-aminothiourea **89** (57%) respectively (**Scheme 3.2-21**). Alkylation of the thione **87** with benzyl chloride **54** afforded thioether-linked dihydroimidazole **88** in 66% yield after column chromatography but the mass return from recrystallization was very poor (3% final yield). Ring closure of the β-aminothiourea **89** using iodomethane gave the amino-linked dihydroimidazole **90** in near quantitative yield before purification. Attempts to purify this product using either a cationic ion exchange resin (Biotage, SCX-2 cartridge) or reverse phase HPLC (MDAP) resulted in very poor yield. Higher overall yield was obtained by not purifying **90** prior to removal of the Boc-protecting group; final compound **91** was then obtained in 47% yield after purification by reverse phase HPLC**.**



Scheme 3.2-21*: a) $SOCl_2$, MeOH, rt, 16 h, 100%; b) conc. aq. NH_3, rt, 16 h, 58%; c) $NaBH_4$, I_2, anh. THF, reflux, 18 h; d) $LiAlH_4$, anh. THF, reflux, 4 h; e) CS_2, NEt_3, EtOH, H_2O, 60 °C, 5 h, then conc. HCl, reflux, 14 h, 31%, ; f)* ***54****, K_2CO_3, acetone, reflux, 17 h, 66%, g)* ***77****, THF, rt, 18 h, 57%, h) iodomethane, acetone, rt, 18 h, 99% (crude); i) TFA, DCM, rt, 2 h, 47%.*

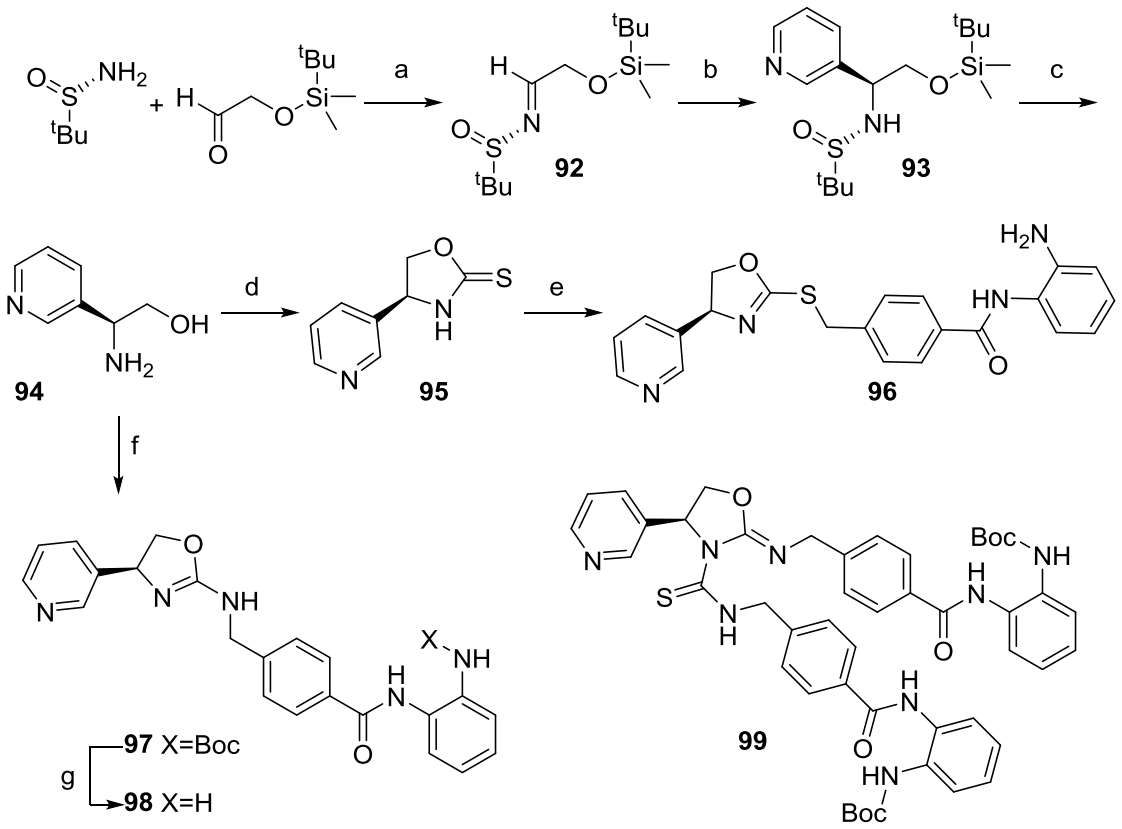
3.2.4 4-(3-Pyridyl)-dihydrooxazoles

Despite being more potent than mocetinostat in the fluorometric assay against HDAC3, the phenyl-substituted amino-linked dihydrooxazole **(*S*)-66b** and amino-linked dihydrothiazole **(*S*)-59b** were less potent in the cancer cell line data (see **Table 3.3-7**). Whilst HDAC3 might not be the primary target in the cancer cell lines tested, mocetinostat seemed to have a cellular effect which was not accounted for by its enzyme inhibition data. The presence of the terminal pyridine ring in mocetinostat could be important in contributing to its inhibitory activity in cells, either by increasing aqueous solubility or by increasing cellular uptake via some sort of cell transport system. In order to establish whether a pyridine ring would impart increased enzymatic inhibition, or cellular activity, the 3-pyridyl substituted dihydrooxazoles were targeted. It was anticipated that if the 3-pyridyl substituted amino alcohol **94** could be prepared, the *S*- and amino-linked dihydrooxazoles would rapidly accessed using the convergent methodology developed above (Scheme 3.2-4 and **Scheme 3.2-19**). Synthesis of the chiral amino alcohol **94** was completed using a sulfinamide chiral auxiliary (**Scheme 3.2-22**). Imine **92** was made from the condensation of (*S*)-2-methylpropane-2-sulfinamide and 2-((*tert*-butyldimethylsilyl)oxy)acetaldehyde using copper(II) sulfate as

a dehydration reagent. Addition of 3-pyridyl lithium (prepared from the lithiation of 3-bromopyridine) to the chiral imine **92** gave protected chiral amino alcohol **93** as a single diastereomer in 33% yield. Finally double deprotection using hydrochloric acid in dioxane afforded chiral amino alcohol **94** in 98% yield.

Formation of the oxazolidine-2-thione **95** using carbon disulfide, and subsequent alkylation of this thione with **54** proceeded uneventfully, albeit with low yield (14% and 45%), to give thioether-linked dihydrooxazole **96**.[122]

Reaction of chiral amino alcohol **94** with isothiocyanate **77** unexpectedly gave double addition product **99**. This product formed as a result of there being excess isothiocyanate **77** present during the second step of the reaction, that is when iodomethane is added and the dihydrooxazole ring is formed. Usually, once the 2-amino-4,5-dihydrooxazole ring is formed, it is immediately protonated by the hydrogen iodide produced in the reaction. However in this case the pyridine ring has a similar basicity to the 2-amino-4,5-dihydrooxazole ring which means that there will be some of the free base of the dihydrooxazole ring present. The excess isothiocyanate **77** then reacted with **97** formed in the reaction to give **99**. This problem was solved by using 1.3 equivalents of amino alcohol **94** to ensure all of the isothiocyanate had been consumed in the first step of the reaction. In this way the desired product **97** was obtained in low yield (21%), and subsequently the Boc-protecting group was removed to give **98** in 60% yield.



Scheme 3.2-22*: a) $CuSO_4$, DCM, rt, 90 h, 42%; b) 3-bromopyridine, BuLi, anh. toluene -78 °C, 1 h, then* ***92****, -78 °C, 5 h, 38%; c) 4M HCl in dioxane, rt, 5 h, 98%; d) CS_2, K_2CO_3, EtOH, H_2O_2, 50 °C, 1 min, 14%; e)* ***54****, K_2CO_3, acetone, reflux, 17 h , 45%; f)* ***77****, THF, MeOH, rt, 17 h, then iodomethane, acetone, rt, 5 h, 21%; g) TFA, DCM, rt, 1.5 h, 60%.*[122]

3.3 Assay results

3.3.1 *In vitro* enzyme assay

All final compounds, those which contain the *N*-(2-aminophenyl)benzamide moiety, were evaluated for inhibition of HDAC activity. The results for the dihydroimidazolones are shown in **Table 3.3-1**; most of the compounds of this ring system showed slight selectivity for HDAC3-NCoR1. The Boc-protected compound **29b** is surprisingly potent; it had been thought that the *t*-butyl carbamate group would be too bulky to sit in the entrance to the tunnel. However presumably the group extends beyond the rim of the tunnel and forms a hydrophobic interaction with residues on the surface of the protein. The phenyl substituted compound **30c** is the most potent compound of the series; it showed at least as great potency as did mocetinostat against HDAC3-NCoR1 and was more selective for this isoform. It is interesting to note that **30c** most closely resembles mocetinostat since it has a direct linkage from the

heterocycle to the terminal aromatic ring, indicating that this is the optimal overall length for HDAC3 inhibition.

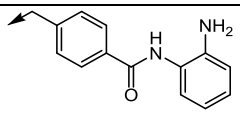
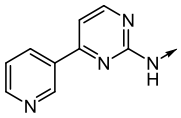
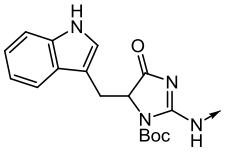
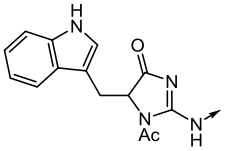
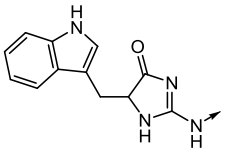
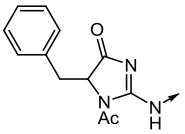
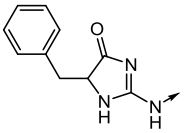
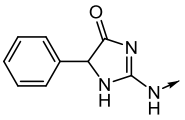
		HDAC inhibitory activity IC_{50} (μM)				
No.	**Structure**	**HDAC1**	**HDAC2**	**HDAC3-NCoR1**	**HDAC6**	**HDAC8**
mocetinostat		0.098±0.06	0.022±0.003	0.022±0.001	>20	35.0±4.4
29b		0.50±0.07	0.048±0.006	0.040±0.002	>20	27.2±1.0
24b		*	0.12±0.02	0.070±0.01	>20	24.3±2.0
30b		0.19±0.06	0.078±0.007	0.104±0.02	>20	>20
24a		*	0.26±0.02	0.083±0.01	>20	22.7±2.7
30a		*	0.066±0.003	0.030±0.006	>20	13.0±2.8
30c		0.35±0.2	0.056±0.01	0.014±0.007	>20	8.0±1.3

Table 3.3-1*: Inhibition of purified recombinant HDAC enzymes. * not determined, >20: less than 45% inhibition at 20 μM. Data is from duplicate experiments and ± standard error measurement of the IC_{50} determination.*[98]

The thioether-linked dihydrothiazoles were evaluated against HDAC2, HDAC3-NCoR1, HDAC6 and HDAC8 (**Table 3.3-2**). Whilst none of the compounds were more potent than mocetinostat against any of these enzymes, once again the phenyl substituted analogue **51b** was the most potent of the series against HDAC3-NCoR1. The results demonstrated that phenyl substituted thiazoline **51b** has increased selectivity for HDAC3 over HDAC2, compared with the phenyl substituted dihydroimidazolone **30c** (7 fold *cf.* 4 fold), which may be a result of testing the single enantiomer of **51b** compared with the racemate of **30c**.

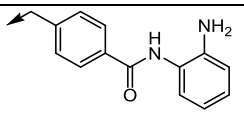
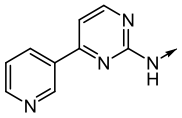
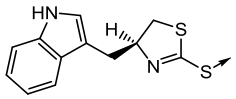
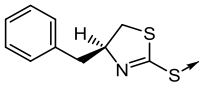
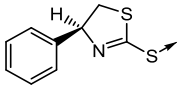
		HDAC inhibitory activity IC_{50} (μM)			
No.	**Structure**	**HDAC2**	**HDAC3-NCoR1**	**HDAC6**	**HDAC8**
mocetinostat		0.022±0.003	0.022±0.001	>20	35.0±4.4
51c		0.92±0.45	0.89±0.24	>20	>20
51a		0.58±0.12	0.509±0.02	>20	>20
51b		0.44±0.06	0.063±0.03	>20	>20

Table 3.3-2*: Inhibition of purified recombinant HDAC enzymes. >20: less than 45% inhibition at 20 μM,. Data is from at least duplicate experiments and ± standard error measurement of the IC_{50} determination.*[98]

Inhibition data for the thioether-linked dihydrooxazoles (**Table 3.3-3**) again revealed enhanced selectivity for HDAC3 compared with mocetinostat, although none showed greater potency than mocetinostat. In this ring system the phenyl substituent **52b** is not significantly more potent against HDAC3 than the benzyl compound **52a**, although **52b** is more selective for HDAC3. Replacing the phenyl ring with a 3-pyridyl ring (as is found in mocetinostat) slightly increases potency against HDAC3 and actually further increases selectivity for HDAC3 against HDAC2, **96** being almost 11 fold selective for HDAC3. Having two nitrogen atoms in the heterocycle results in greater HDAC3 inhibition; thioether-linked dihydroimidazole **88** is the most potent thioether-linked compound prepared and is of comparable potency to mocetinostat.

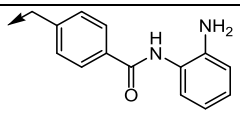
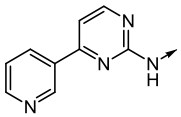
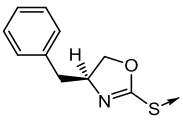
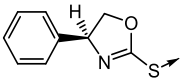
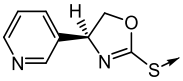
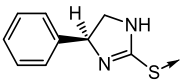
		HDAC inhibitory activity IC_{50} (μM)				
No.	**Structure**	**HDAC1**	**HDAC2**	**HDAC3-NCoR2**	**HDAC6**	**HDAC8**
mocetinostat		0.098±0.06	0.022±0.003	0.045±0.01	#68%	35.0±4.4
52a		0.70±0.04	0.25±0.07	0.071±0.006	#60%	88.0±11
52b		1.2±0.6	0.36±0.05	0.066±0.008	#54%	9.5±2.2
96		◊13%	0.61±0.13	0.056±0.003	#62%	112±14
88		0.38±0.005	0.18±0.006	0.038±0.002	#64%	14.3±1.8

Table 3.3-3*: Inhibition of purified recombinant HDAC enzymes. # percentage inhibition at 20 μM, ◊ percentage inhibition at 0.2 μM. Data is from at least duplicate experiments and ± standard error measurement of the IC_{50} determination.*[98,122]

As expected the amino-linked dihydrothiazoles (**Table 3.3-4**) exhibited much more potent inhibition than the thioether-linked analogues above (**Table 3.3-2**). A few compounds **(*S*)-59b**, **(*S*)-59d**, and **59e**) were at least as potent as mocetinostat against HDAC3-NCoR1 and all displayed enhanced selectivity towards that isoform. Although phenyl, 4-hydroxyphenyl, and benzyl mono-substitution at both the 4- and 5-positions of the heterocycle consistently gave compounds with good potency, bulkier 4,5-diphenyl substitution of this ring system was detrimental to inhibition of all HDAC isoforms tested. This could be because of a general size requirement to fit in the entrance of the active site tunnel, although this seems unlikely given that bulky Boc-protected dihydroimidazolone **29b** is a potent HDAC3 inhibitor (**Table 3.3-1**). Alternatively it could be the *trans*-nature of the two phenyl rings causing loss of inhibition, because it is impossible for both rings to make contacts with the surface of the protein. Therefore one ring must be angled into the solvent which creates unfavourable ordering of water molecules around the hydrophobic ring.

It is interesting to note that **(*S*)-59b** and its enantiomer **(*R*)-59b** have different selectivity profiles. The (*S*)-enantiomer **(*S*)-59b** is moderately HDAC3 selective whereas the (*R*)-enantiomer **(*R*)-59b** is slightly HDAC2 selective, although it has a greatly reduced selectivity. The two compounds exhibit the following HDAC1:2:3 selectivity profiles **(*S*)-59b**; 77:7:1 and **(*R*)-59b**; 6:1:1.4. The 4-benzyl and 5-phenyl analogues do not show a similar switch in selectivity between enantiomers; in these

cases the selectivity profile within each pair of enantiomers is very similar.

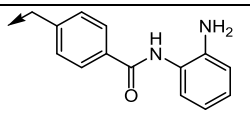
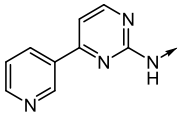
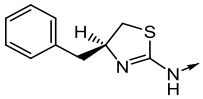
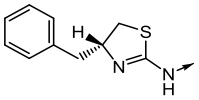
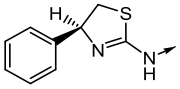
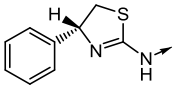
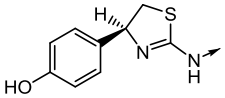
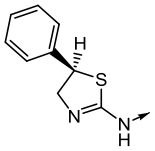
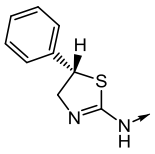
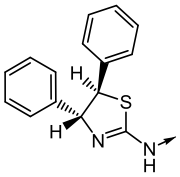
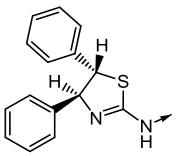
		HDAC inhibitory activity IC_{50} (μM)				
No.	**Structure**	**HDAC1**	**HDAC2**	**HDAC3-NCoR1**	**HDAC6**	**HDAC8**
mocetinostat		0.098±0.06	0.022±0.003	0.022±0.001	>20	35.0±4.4
(*S*)-59a		0.92±0.32	0.104±0.017	0.052±0.003	>20	34.9±7.1
(*R*)-59a		0.41±0.14	0.082±0.026	0.036±0.006	>20	12.7±2.5
(*S*)-59b		0.93±0.30	0.085±0.015	0.012±0.008	>20	4.1±0.85
(*R*)-59b		0.37±0.06	0.056±0.007	0.079±0.01	>20	10.7±1.1
59e		0.18±0.06	0.044±0.005	0.016±0.005	*	15.0±1.8
(*R*)-59d		*	0.111±0.008	0.023±0.009	*	1.4±0.1
(*S*)-59d		0.55±0.23	0.081±0.003	0.012±0.01	*	2.3±0.2
(*R*,*R*)-59f		*	0.425±0.074	0.287±0.123	*	14.9±0.5
(*S*,*S*)-59f		*	0.538±0.101	0.388±0.216	*	12.6±2.1

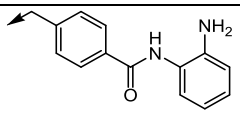
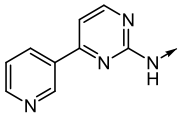
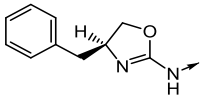
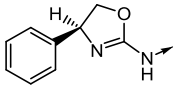
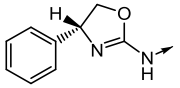
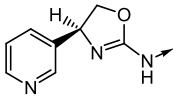
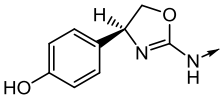
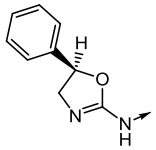
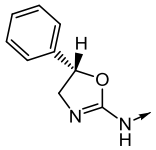
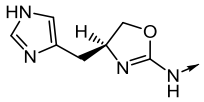
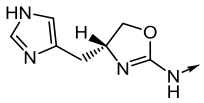
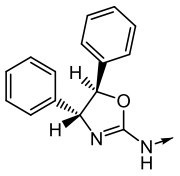
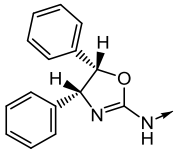
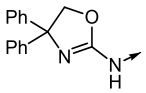
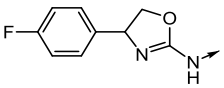
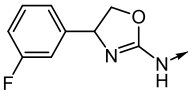
***Table 3.3-4**: Inhibition of purified recombinant HDAC enzymes. >20: less than 45% inhibition at 20 μM, * not determined. Data is from duplicate experiments and ± standard error measurement of the IC_{50} determination.*[98]

Evaluation of amino-linked dihydrooxazoles against HDAC3-NCoR2 revealed that they were all at least as potent or more than mocetinostat with two exceptions, 4,4-diphenyl and trans-4,5-diphenyl substituted compounds **66g** and **(*R*,*R*)-66f**, which were less potent (**Table 3.3-5**). Trends similar to those seen for the thioether-linked

dihydrooxazoles can be seen again in these amino-linked analogues, although to a slightly lesser extent since the overall potency is greater with the amino-linked series. The phenyl substituted compound (**(*S*)-66b**) is slightly more potent and selective for HDAC3 than the benzyl substituted analogue (**66a**), and the 3-pyridyl version (**98**) is again slightly more potent and selective for HDAC3 than phenyl. Changes to either the nature or the stereochemistry of the terminal ring have a less significant impact on HDAC3 potency, and overall isoform selectivity, than they did in the corresponding dihydrothiazole series. Fluoro-substituents at either the *para*- or *meta*-position of the 4-phenyl compound are tolerated (**66i** and **66j**), as are *para*-trifluoromethyl and *para*-hydroxy substituents, which means there is a capability to easily adjust physicochemical properties without greatly affecting enzyme inhibition. Indeed even the imidazoylmethyl compounds derived from L- and D-histidine (**(*S*)-66h** and **(*R*)-66h**) are potent, low nanomolar HDAC3 inhibitors, and show some of the same isoform selectivity.

Once again *trans*-4,5-diphenyl substitution (**(*R,R*)-66f**) was deleterious for inhibition of HDAC3, and in anticipation that this would be the case, *cis*-4,5-diphenyl amino-linked dihydrooxazole (**(*R,S*)-66f**) was also prepared in the hope that this might retain or improve potency. This was indeed the case **(*R,S*)-66f** is the most potent HDAC3 inhibitor of the series, and retains at least 12 fold selectivity over HDAC1 and HDAC2.

Another point of particular note is that whereas in the analogous dihydrothiazole series the (*S*)-4-phenyl and (*S*)-5-phenyl compounds were the more potent enantiomers against HDAC3 (**(*S*)-59b** *cf.* **(*R*)-59b,** and **(*S*)-59d** *cf.* **(*R*)-59d**, **Table 3.3-4**), in the amino-linked dihydrooxazoles the opposite enantiomers of both the 4- and 5-phenyl substituted compounds were more potent against HDAC3 (**(*R*)-66b** *cf.* **(*S*)-66b**, and **(*R*)-66d** *cf.* **(*S*)-66d**). This indicates that the dihydrooxazole ring might be orientated in a different conformation to the dihydrothiazole ring when bound to HDAC3. Alternatively the change from using HDAC3-NCoR1 to HDAC3-NCoR2 in the assay could be responsible for these differences, since the source of enzyme had to be changed between testing the two different series.

		HDAC inhibitory activity IC_{50} (μM)				
No.	**Structure**	**HDAC1**	**HDAC2**	**HDAC3-NCoR2**	**HDAC6**	**HDAC8**
mocetinostat		0.098±0.06	0.022±0.003	0.045±0.01	#68%	35.0±4.4
(*S*)-66a		0.20±0.02	0.13±0.04	0.041±0.001	#72%	63.0±5.4
(*S*)-66b		0.53±0.03	0.18±0.03	0.040±0.002	#66%	14.7±3.2
(*R*)-66b		0.082±0.002	0.18±0.03	0.033±0.002	#69%	80.7±19
98		0.26±0.01	0.43±0.07	0.031±0.002	#62%	111±15
66e		0.29±0.007	0.23±0.02	0.018±0.001	#13%	83.3±12
(*R*)-66d		0.076±0.005	0.192±0.007	0.011±0.003	#74%	173±38
(*S*)-66d		0.39±0.007	0.094±0.038	0.035±0.002	#72%	8.1±0.88
(*S*)-66h		0.078±0.002	0.16±0.01	0.021±0.001	#67%	179±29
(*R*)-66h		0.13±0.003	0.29±0.008	0.018±0.001	#61%	113±13
(*R*,*R*)-66f		◊28%	0.28±0.03	0.118±0.02	#67%	29.2±2.9
(*R*,*S*)-66f		0.080±0.04	0.11±0.03	0.0063±0.005	#71%	25.2±3.2
66g		◊21%	0.40±0.08	0.055±0.02	#61%	53.5±6.4
66i		0.19±0.009	0.15±0.008	0.034±0.004	#68%	33.9±6.1
66j		0.24±0.003	0.24±0.02	0.024±0.001	#70%	52.2±8.6

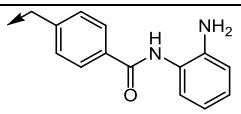
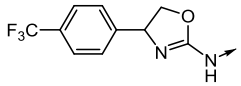
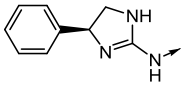
		HDAC inhibitory activity IC_{50} (μM)				
No.	**Structure**	**HDAC1**	**HDAC2**	**HDAC3-NCoR2**	**HDAC6**	**HDAC8**
66k		◊20%	0.34±0.05	0.034±0.002	#67%	82.1±13
91		0.24±0.08	0.27±0.03	0.015±0.001	#78%	5.5±0.3

Table 3.3-5*: Inhibition of purified recombinant HDAC enzymes. # percentage inhibition at 20 μM, ◊ percentage inhibition at 0.2 μM. Data is from at least duplicate experiments and ± standard error measurement of the IC_{50} determination.*[122]

3.3.2 Physicochemical properties

A selection of the final compounds prepared in the project was sent to GSK, Stevenage in order to have some physicochemical properties measured. This data is shown below in **Table 3.3-6** and it is useful to help identify compounds that are likely to be more potent *in vivo*. For example diphenyl-substituted compound **(*R*,S)-66f** which was the most potent compound *in vitro* might be less effective *in vivo*. This is because it has a high percentage human serum albumin (HSA) binding, which means that a large proportion of the compound will be bound to HSA. Other non-specific protein binding is also more likely, and so the concentration of free drug in circulation will effectively be reduced. This occurs when compounds are too lipophilic and is often accompanied by poor aqueous solubility, however **(*R*,S)-66f** has acceptable solubility.

At the other end of the spectrum are analogues **(*S*)-66h** and **(*R*)-66h,** containing an imidazole ring; they show good solubility and low HSA binding, however in this case the artificial membrane permeability is very low. This suggests that these compounds will not rapidly cross cell membranes which would result in lower activities in cell based assays and *in vivo* since HDACs are intracellular targets. The cell membrane is a lipid membrane and because the imidazole ring is basic and protonated at pH 7.4, the charged species cannot easily cross the lipophilic interior of the membrane. This assay uses an artificial membrane to test permeability and sometimes *in vivo* cell permeability can actually be higher than this, possibly as a result of active transport systems.

Several of the compounds tested showed good overall physicochemical properties, indicating that they might be suitable for progressing into cell based assays.

Compound	Chrom log P	Chrom log $D_{7.4}$	Solubility CLND (µg/mL)	Permeablilty (pH 7.4, nm/s)	%HSA binding
Mocetinostat	2.96	2.96	107	350	92.5
(*S*)-59b	4.65	4.24	108	510	
52a	5.67	5.41	31	440	96.5
96	3.32	3.19	108	270	87.5
88	4.31	3.49	116	300	91
66a	4.15	2.86	≥148	230	85.9
(*S*)-66b	3.74	3.10	≥153	330	85.3
(*R*)-66b	3.76	3.09	≥151	310	84.7
98	1.78	1.71	≥124	33	68.7
66e	2.13	1.74	≥130	13	74.4
(*R*)-66d	3.58	2.79	98	180	84.9
(*S*)-66d	3.84	2.99	77	162	89.9
(*S*)-66h	0.95	0.90	≥142	<3	46.8
(*R*)-66h	0.93	0.89	≥158	<3	46.5
(*R*,*R*)-66f	5.41	5.01	6	410	96.9
(*R*,S)-66f	5.01	4.54	73	410	94.9
66g	5.59	5.29	7	390	96.6
66i	3.97	3.39	147	270	88.1
66j	3.84	3.42	≥131	340	88.5
66k	4.72	4.54	8	360	94.3
91	3.46	1.92	≥116	17	72.2

***Table 3.3-6**: Table of physicochemical data measured by GSK, Stevenage.*[122] *Chrom log D and Chrom log P values*[123] *and permeability (artificial membrane permeability)*[124] *were measured using procedures previously described. Solubility was measured using chemiluminescent nitrogen detection (CLND).*[125]*Human serum albumin (HSA) binding was measured using a fast gradient HPLC method with a chemically bonded protein stationary phase.*[126] *Heat map indicates favourable numbers (green) through to unfavourable numbers (red).*

3.3.3 Cancer cell growth inhibition

Initially some cancer cell line testing was carried out by Peter Soden at GSK, Stevenage. A selection of five compounds was tested for their ability to inhibit the growth of four cancer cell lines (**Table 3.3-7**). Mocetinostat and vorinostat were tested as controls in the assay. The results for dihydroimidazolones **30a** and **30c** are disappointing; they both weakly inhibited colon cancer cell line HCT116 but were inactive against the three other cell lines tested. Amino-linked dihydrothiazoles **(*S*)-59b** and **(*S*)-59a** were generally more potent than the thioether-linked dihydrothiazole **51c** except against prostate cancer cell line DU145 which **51c** inhibited almost as well as vorinostat.

	Cell growth inhibition IC_{50} (μM)			
Compound	**A549**	**DU145**	**HCT116**	**MCF-7**
mocetinostat	1.73±0.03	2.06±0.03	0.70±0.04	1.26±0.03
vorinostat	2.27±0.04	2.58±0.02	1.39±0.03	2.29±0.06
30a	>50	>50	16.6±0.22	>50
30c	>50	>50	18.2±0.15	30.1±0.43
(*S*)-59b	5.78±0.03	6.40±0.02	2.17±0.03	5.43±0.09
(*S*)-59a	6.37±0.03	5.45±0.06	1.95±0.05	3.09±0.03
51c	9.53±0.38	2.80±0.10	3.64±0.21	9.91±0.20

Table 3.3-7*: Cell growth inhibition data. Data from three independent experiments.* ** not determined. Data were collected by Peter Soden at GSK, Stevenage.*[98]

3.4 Conclusion

Highly convergent methodology that enabled the rapid synthesis of chiral mocetinostat analogues was developed, whereby the pyrimidine ring is replaced by a non-aromatic 5-membered heterocyclic ring. Multiple series of compounds were prepared which contain a different chiral heterocycles, as well as different terminal aromatic capping groups. *In vitro* testing against individual isoforms revealed several compounds that are more potent and selective inhibitors than mocetinostat against HDAC3. In all ring systems, those compounds containing a 2-amino-linker are more potent than the thioether-linked equivalent. Of the heterocycles prepared both the dihydrooxazole and the dihydroimidazole showed the highest levels of inhibition. Additionally, many of the new HDAC inhibitors displayed higher solubility and lower binding to human serum albumin than that of mocetinostat. In an *ex vivo* cancer cell growth assay, some of the new compounds that were tested showed low micro molar inhibition of the cancer cell lines, but the level of inhibition was inferior to mocetinostat in all cases.

4 Search for novel zinc-binding groups

4.1 Fragment screen

Recently there have been several HDAC inhibitors discovered which contain novel zinc-binding groups (see section 1.4.1, p 19). Discovery of a new zinc-binding group for HDAC inhibitors would be an important discovery, especially if that zinc-binding group had inherent isoform selectivity. A selection of fragments that were commercially available or easy to prepare was chosen from potential bi-dentate metal binding groups. The computational study by Chen *et al.*[55] (discussed in section 1.4.1) was used to guide the choice of fragments tested. In particular those fragments which were amenable to further functionalisation were selected. The 8-hydroxyquinoline had shown steric clashes upon docking into the full structure of HDAC2 by Chen *et al.*, but it was included in the set to be tested since it might not have steric clashes with another isoform which would achieve the desired aim of isoform selectivity.

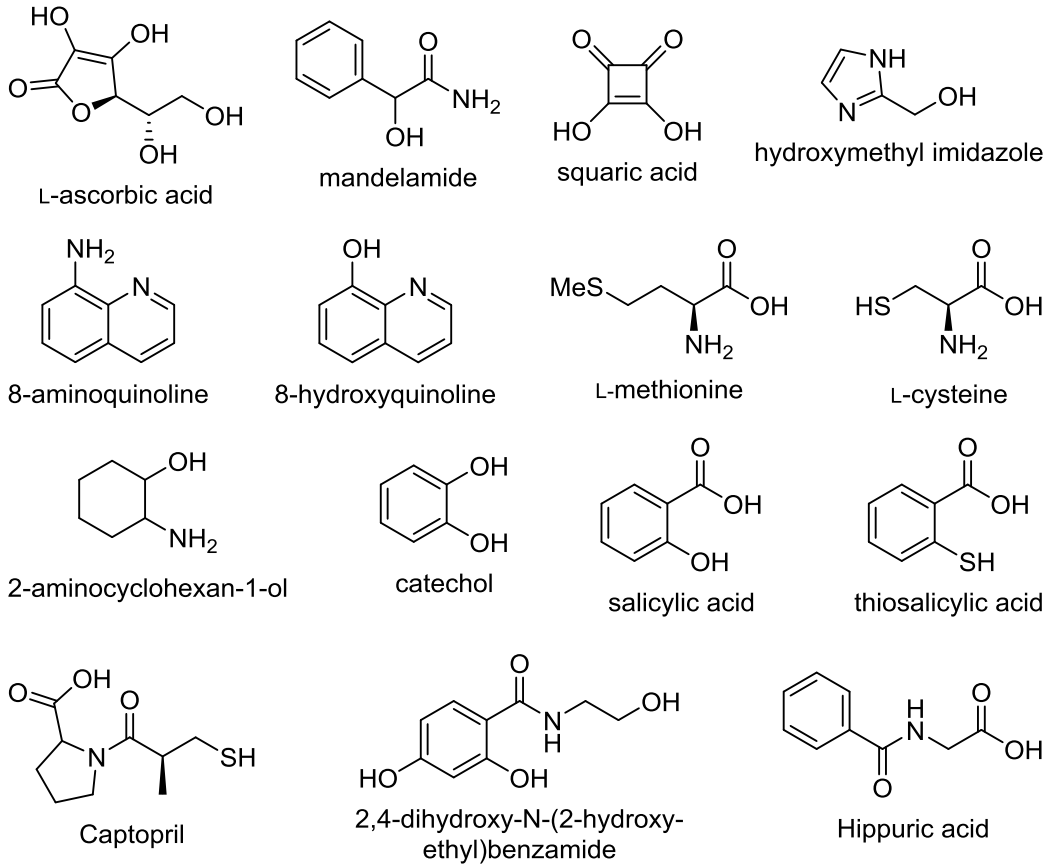
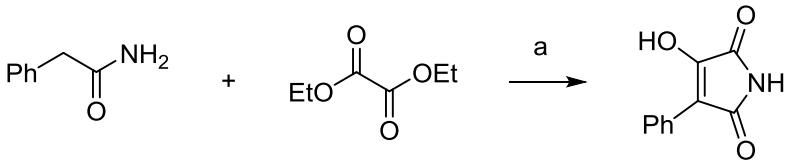


***Figure 4.1-1**: Commercially available fragments tested.*

Only one fragment, 2-hydroxy-3-phenyl maleimide **100** was synthesised. Following a procedure from Xu *et al.*;[127] diethyl oxalate was reacted with 2-phenylacetamide and potassium *tert*-butoxide in THF to afford the phenyl maleimide **100** in very poor yield (4%). This compound was actually a false positive in the assay; it appeared to be a potent inhibitor of HDAC8 but it was in fact interfering with the assay. This was confirmed by carrying out an experiment using deacetylated standard **130** (see section 5.1); the fluorescence was reduced to 10% of the control, when **100** (1 mM) was added before or after trypsin. This suggested **100** has significant UV absorption at the wavelength of detection (460 nM) and because of this there is no inhibition data shown for this compound.



100

***Scheme 4.1-1**: a) t-BuOK, THF, 0 °C, 1.5 h, 4%.*

Fragment	HDAC1	HDAC2	HDAC3-NCoR1	HDAC8
L-ascorbic acid	22	21	*	42
mandelamide	20	20	*	-3
squaric acid	17	19	32	6
2-hydroxymethyl imidazole	27	22	*	4
8-aminoquinoline	43	**60**	**66**	**76**
8-hydroxyquinoline	39	42	**57**	**91**
L-methionine	-4	2	-7	3
L-cysteine	-9	4	-10	**79**
2-aminocyclohexan-1-ol	*	*	32	-5
catechol	*	*	**96**	**100**
salicylic acid	*	*	38	14
thiosalicylic acid	48	17	35	**69**
captopril	29	15	38	30
2,4-dihydroxy-*N*-(2-hydroxyethyl)benzamide	20	21	29	6
hippuric acid	32	12	33	6

***Table 4.1-1**: Percentage inhibition at 1 mM. All data is averaged from duplicate experiments except HDAC3-NCor1 which is from a single experiment. * not determined.*

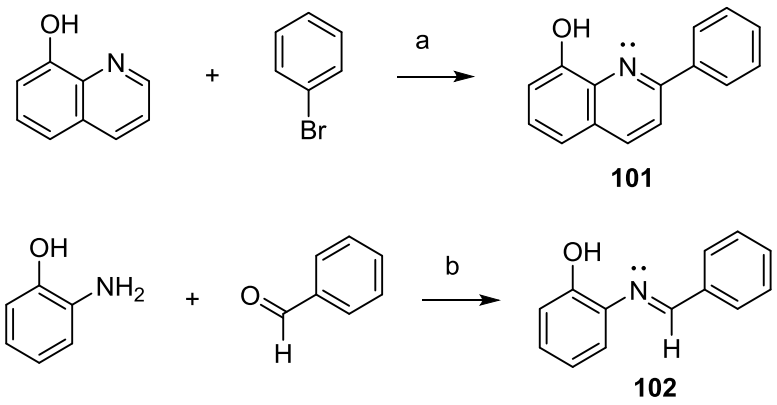
From **Table 4.1-1** it can be seen that of all the fragments tested only 8-aminoquinoline, 8-hydroxyquinoline, catechol, L-cysteine and thiosalicylic acid had >50% inhibition against any isoform. Therefore a dose response curve was measured for these compounds against HDAC8 and the resulting IC_{50} values are shown below in **Table 4.1-2**. HDAC8 was chosen since that was shown to be the most sensitive of the isoforms tested of the initial fragment assay

8-aminoquinoline	8-hydroxyquinoline	catechol	L-cysteine	thiosalicylic acid
436	96.3	214	265	223

***Table 4.1-2**: IC_{50} values (μM) against HDAC8 from a single experiment.*

4.2 Substituted 8-hydroxyquinolines

The 8-hydroxyquinoline seemed to be the most promising fragment in terms of novelty, ability to be developed and it was the most potent HDAC8 fragment from the full dose-response experiment. A simple phenyl-substituted 8-hydroxyquinoline **101** was prepared according to a literature procedure,[128] in order to establish some very basic SAR of this novel zinc-binding group. The nature of the nitrogen atom is presumably vital for coordination to zinc because its lone pair is orthogonal to, and not delocalised around the aromatic ring, therefore the electrons are freely available to form a bond with the zinc ion. A nitrogen atom with similar electronics is found in an *N*-phenyl Schiff base **102** (**Scheme 4.2-1**). The delocalisation of electrons over the two aromatic rings effectively locks the conformation so that nitrogen's lone pair is orientated in the same way as it is in the quinoline. Therefore the *N*-(2-hydroxyphenyl) Schiff base **102** was prepared according to a literature procedure,[129] to test whether the quinoline could be replaced by a *N*-(2-hydroxyphenyl) Schiff base, since that would make analogue synthesis easier and faster than with the quinoline.



***Scheme 4.2-1**: a) Li, anh. Et_2O, reflux, 1 h, then rt, air, 2 h, 70%; b) MeOH, reflux, 3 h, 37%.*

Extension of the 8-hydroxyquinoline scaffold in this direction had failed to maintain inhibition, **101** and **102** do not inhibit HDAC8 (see 4.2.1, p 94), so it was decided to explore the opposite direction, that is 7-substitution instead of 2-substitution. It was decided to target substituents which had a SAHA like flexible linker and cap group. A route was devised that would give access to a selection of analogues (**Figure 4.2-1**) including those with an unsaturated linker, and the corresponding 8-methoxyquinolines.

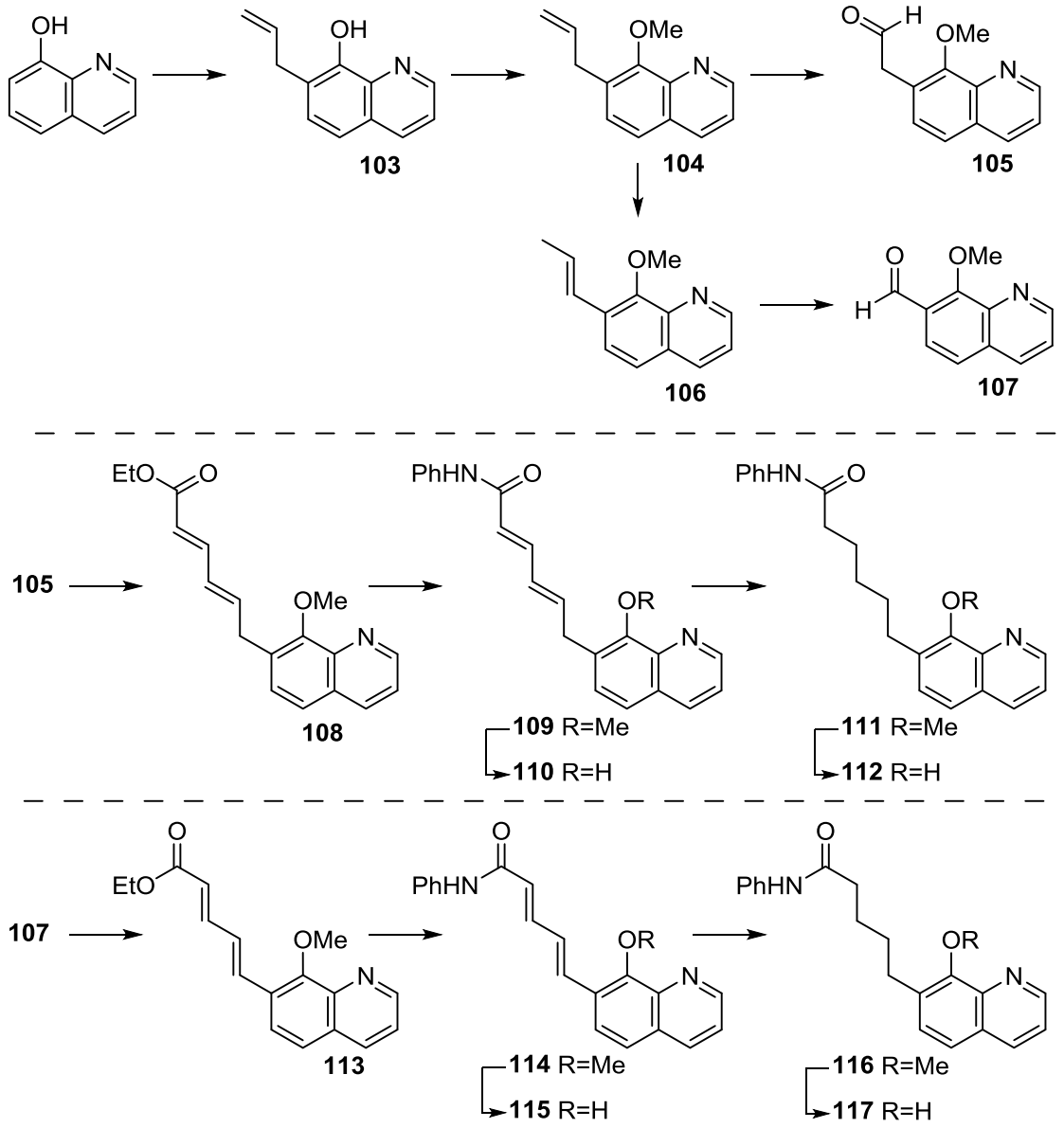
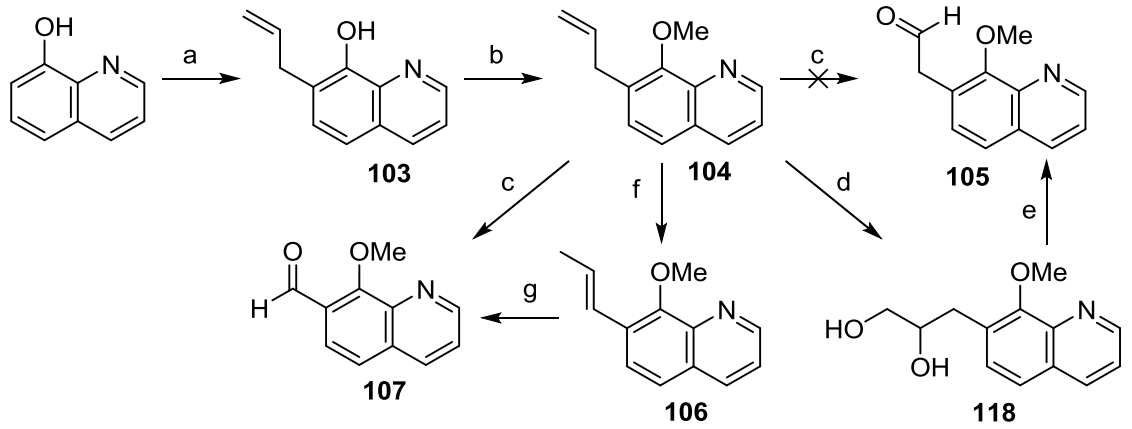


***Figure 4.2-1**: Proposed route to SAHA like 8-hydroxyquinolines.*

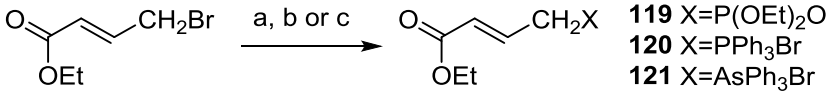
Synthesis of the SAHA like 8-hydroxyquinolines did not proceed as smoothly as planned. The allylation and Claisen rearrangement successfully installed the allyl group at the required 7-position and subsequent protection of the phenol as a methyl ether gave **104** in very good yield on a gram scale. Attempts to oxidatively cleave the alkene using osmium tetroxide and sodium metaperiodate gave aryl aldehyde **107** in 87% yield instead of acetaldehyde **105**. This was because the enol tautomer of desired product **105** was forming in the reaction and then undergoing a second dihydroxylation by osmium tetroxide to give a 1,2-diol intermediate which could then be oxidatively cleaved by excess sodium metaperiodate giving aldehyde **107**. The desired aldehyde was obtained by separating the two steps; reaction of **104** with osmium tetroxide using potassium ferricyanide as a co-oxidant gave the 1,2-diol **118**. The diol was subsequently oxidatively cleaved using sodium metaperiodate to give unstable acetaldehyde **105** in

66% yield over 2 steps. Aryl aldehyde **107** could also be accessed by rearrangement of the alkene, using potassium *tert*-butoxide, and then oxidative cleavage of the new olefin, using osmium tetroxide and sodium metaperiodate, in similar overall yield (89%) to the one step process.



***Scheme 4.2-2**: a) i) allyl bromide, K_2CO_3, acetone, reflux, 24 h; ii) neat, 200 °C, 30 min, 92%; b) iodomethane, NaOH, THF, rt, 18 h, 100%; c) OsO_4, $NaIO_4$, dioxane, H_2O, rt, 16 h, 87% (**107**); d) OsO_4, $K_3Fe(CN)_6$, t-BuOH, H_2O, rt, 24 h, 81%; e) $NaIO_4$, THF, H_2O, rt, 5 min, 81%; f) t-BuOK, anh. THF, rt, 2 h, 94%; g) OsO_4, $NaIO_4$, dioxane, H_2O, rt, 16 h, 95%.*

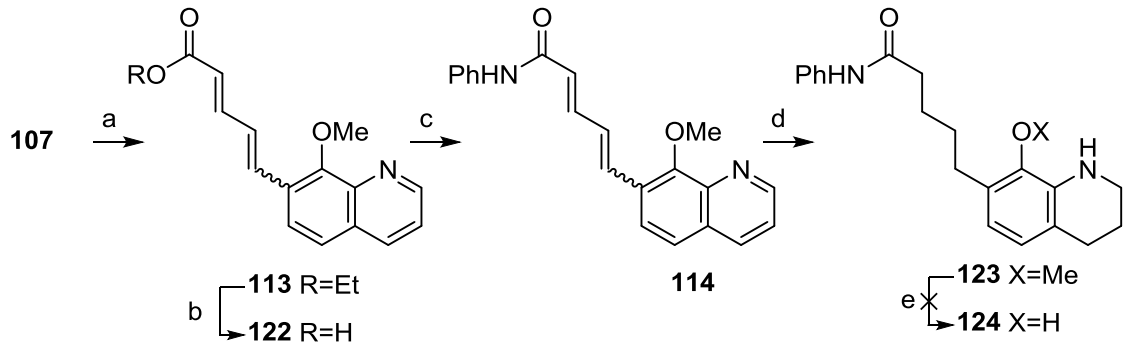
It was anticipated that a Horner-Wadsworth-Emmons reaction of phosphonate **119** with both aldehydes **107** and **105** would give the desired (*E*, *E*) dienes.



***Scheme 4.2-3**: a) $P(OEt)_3$, 130 °C, 4 h, 84% (**119**); b) PPh_3, toluene, rt, 16 h, 74% (**120**), c) $AsPh_3$, toluene, reflux, 1.5 h, rt, 7 days, 75% (**121**).*

However reaction of **107** with **119** and sodium hydride in 1,2-dimethoxyethane gave only ~20% conversion by NMR and starting material aldehyde was still present. The product **113** could not be separated because it has the same R_f as the aldehyde starting materials. Therefore triphenylphosphonium bromide salt **120** was prepared (**Scheme 4.2-3**-b) then subsequently deprotonated using potassium, *tert*-butoxide and reacted with aldehyde **107** to give **113** (**Scheme 4.2-4**). However despite being a stabilised ylid which should give (*E*)-alkene, the product was roughly a 1:1 mixture of (*E*):(Z) isomers. Instead of purifying the ester the crude mixture was treated with lithium hydroxide to give the carboxylic acid **122** which was purified by a simple basic aqueous extraction procedure. Carboxylic acid **122** was converted into the acid chloride using oxalyl chloride, and coupled with aniline to afford the anilide **114** which was still a mixture of geometric isomers. Hydrogenation of the diene was attempted using a

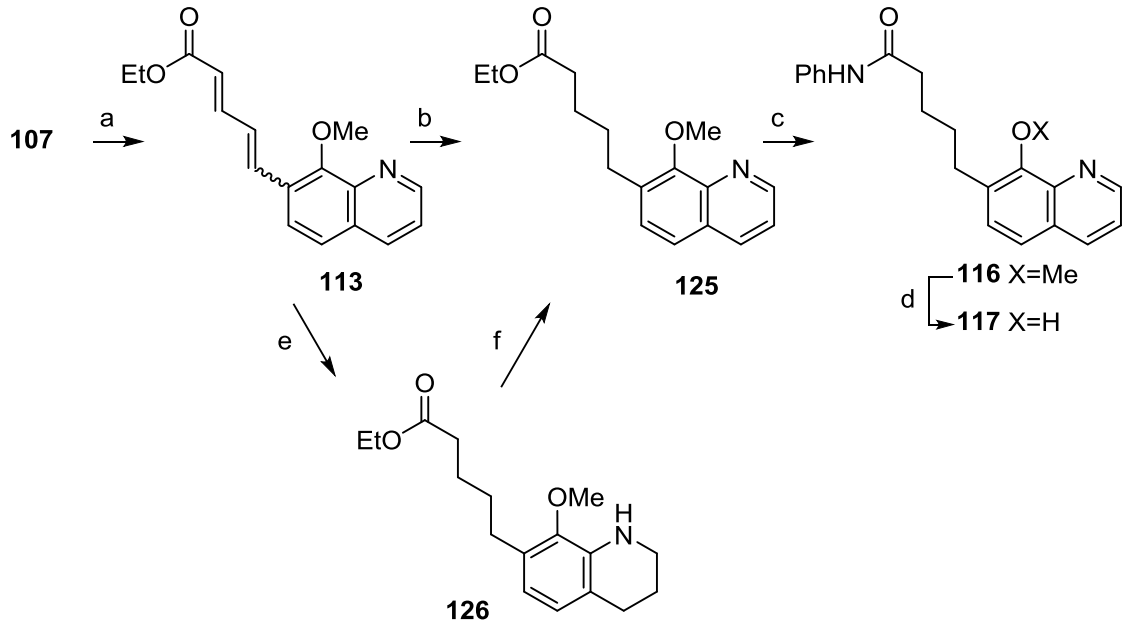
hydrogen balloon and palladium on carbon, in a mixture of dichloromethane and methanol because **114** was insoluble in only dichloromethane. None of the desired quinoline **116** was isolated; instead the pyridine ring had also been reduced to give tetrahydroquinoline **123** in good yield (84%). It was not expected that the 8-hydroxytetrahydroquinoline ring would be a good zinc-binding group, for the reasons discussed at the beginning of this section, but demethylation of **123** was attempted using boron tribromide. The reaction appeared to work by TLC however during column chromatography fractions containing the product became grey/black in colour which persisted after evaporation of the solvent. The proton NMR spectrum of the product was very broad and could not confirm that it was the desired compound **124**; it appears that there may have been some sort of metal contaminant, unless the product is just very unstable.



***Scheme 4.2-4**: a) **120**, t-BuOK, anh. THF, rt, 1 h, then **107**, rt, 1 h; b) LiOH, THF, H_2O, reflux, 15 h, 59% (2 steps); c) i) oxalyl chloride, DCM, DMF, rt, 1 h, reflux, 1 h; ii) aniline, pyridine, DCM, rt, 16 h, 38%; d) H_2, 10% Pd/C, DCM, MeOH, 48 h, 84%; e) BBr_3, DCM, -78 °C, 4 h.*

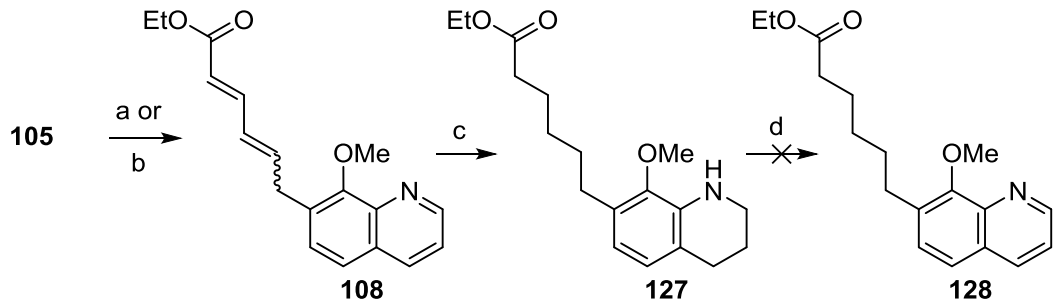
It is known that stabilised triphenylarsonium ylids give good *trans*-selectivity in Wittig reactions and are more reactive than the corresponding phosphonium ylid,[130,131] so the corresponding triphenylarsonium salt **121** was prepared (**Scheme 4.2-3**-c). Reaction of this salt (using *n*-butyllithium to form the ylid) with aldehyde **107** gave ester **113** in 75% yield, also as an inseparable mixture of geometrical isomers. Since it was not possible to obtain the *E,E* diene selectively, only the saturated analogue would now be sought. Therefore reduction of the alkenes was attempted using a hydrogen balloon and palladium on carbon in ethyl acetate. It was expected that the over-reduction experienced previously (**Scheme 4.2-4**-d) would not occur in a non-protic solvent. However, even when carrying out the hydrogenation in ethyl acetate some of the quinoline was reduced before the alkenes had been fully reduced, accounting for the low yield of **125** (24%). An alternative would be to carry out complete over-reduction followed by re-oxidation to the quinoline, which the literature suggested could be

accomplished using 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ).[132] In order to test this alternative route, complete reduction of **113** gave **126** in 93% yield, however re-oxidation to the quinoline with DDQ was not a clean reaction and **125** was obtained in low yield (15%). The anilide **116** was prepared in good yield directly from ethyl ester **125** using Weinreb conditions;[133] that is initially treating aniline with trimethylaluminium to make the dimethylaluminium anilide and then addition of ester **125**. Demethylation of **116** was successfully completed using boron tribromide to afford the desired 8-hydroxyquinoline **117** in 49% yield.



Scheme 4.2-5*: a)* ***121****,* ^{n}*BuLi, anh. THF, rt, 16 h, 75%, b)* H_2*, 10% Pd/C, EtOAc, rt, 5 h, 24%, c) aniline,* $AlMe_3$*, anh. toluene, rt, 17 h, 61%; d)* BBr_3*, DCM, -10 °C to rt, 2 h, 49%; e)* H_2*,10% Pd/C, MeOH, rt, 20 h, 93%; f) DDQ, DCM, rt, 2.5 h, 15%.*

Reaction of diethyl phosphonate **119** with the more reactive aldehyde **105**, did give the diene product **108** (**Scheme 4.2-6**), but as an inseparable mixture of geometrical isomers, and in low yield (19%). Using triphenylarsonium salt **121** the yield was improved (35%) but an *E*:*Z* mixture was still obtained. Complete reduction of diene **108** using hydrogen and palladium on carbon, in methanol, gave tetrahydroquinoline **127** in quantitative yield. However subsequent DDQ oxidation to the quinoline was unsuccessful. Due to time constraints no further attempts were made to prepare this compound. In order to access the unsaturated *E,E*-diene, olefin metathesis might be a more successful approach, however the problem of how to reduce the alkenes without reducing the quinoline remains.



***Scheme 4.2-6**: a) **119**, NaH, anh. THF, rt, 12 h, 19%; b) **121**, ^{n}BuLi, anh.l THF, rt 16 h, 35%; c) H_2, 10% Pd/C, MeOH, rt, 20 h, 100%; d) DDQ, DCM, rt, 2 h.*

4.2.1 Enzyme assay results

The substituted 8-hydroxyquinolines and 8-methoxyquinolines were tested for inhibition of HDAC8, however none of the compounds were potent inhibitors and so an IC_{50} value could not be generated for any of the compounds. **Table 4.2-1** below shows the percentage inhibition of HDAC8 by each compound at 1 mM, the values are all significantly lower than the unsubstituted fragment 8-hydroxyquinoline (**Table 4.1-2**, 91%).

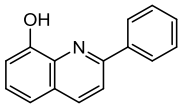
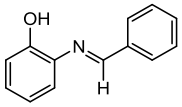
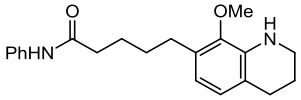
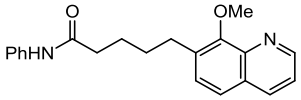
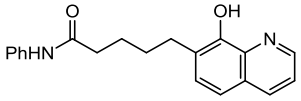
Compound	Structure	% inhibition
101		21
102		19
123		-6
116		34
117		3

***Table 4.2-1**: Percentage inhibition of HDAC8 at 1 mM average values from duplicate experiments except **101** and **102** which are from a single experiment.*

No further exploration of the 8-hydroxyquinoline zinc-binding group was carried out; the question of whether the 8-hydroxyquinoline ring can be extended to give increased potency against HDAC8 remains unanswered, although the substitutions at the 2- and 7- positions made in this project were severely detrimental to activity. It seems unlikely therefore that any substitution at the 2- or 7- position will furnish

compounds with increased potency. Substitution at the 3- or 6- position might be more successful; however the synthesis of these compounds is less facile.

4.3 Conclusion

An investigation was carried out, to find novel zinc-binding groups that could give rise to new, more selective HDAC inhibitors. Initial screening of a targeted set of zinc-binding fragments, demonstrated that the 8-hydroxquinoline was the most promising of those tested, against HDAC8. Subsequently a set of substituted 8-hydroxquinolines was synthesised, with substituents at the 2- or 7-positions of the quinoline ring. However all of the extended 8-hydroxquinolines that were made showed no significant inhibition of HDAC8. More work is needed to fully understand the SAR of the 8-hydroxquinoline fragment, including substituents at other positions around the ring system, to determine whether it could be useful as a new zinc-binding group with enhanced isoform selectivity.

5 *In vitro* deacetylation assays

Initially, *in vitro* testing of compounds was carried out by Scottish Biomedical, UK. A selection of compounds, including vorinostat and mocetinostat as controls, was sent to be tested against HDAC11 to determine IC_{50} values. Vorinostat showed strong inhibition, yet the IC_{50} value calculated deviated significantly from literature values reported (26.6 nM *cf.* 100[53]-362[134,135] nM). Furthermore mocetinostat was found to be completely inactive against HDAC11, which is in stark contrast with the literature report that it has an IC_{50} value of 0.59 μM.[68] Since assay results from outsourcing did not agree with literature values, an enzyme assay was developed in order to evaluate the inhibition of compounds without the need for an external company.

5.1 End-point assay

Purified recombinant HDAC11 was purchased from Enzo Life Sciences, together with an assay kit containing fluorescent substrate for the deacetylation reaction, Fluor-de-lys® SIRT1. This acetylated substrate is deacetylated by HDAC11 in the assay (**Figure 5.1-1**), deacetylation activates the substrate for a second step. A developer solution is added which selectively releases the fluorophore from the deacetylated substrate. The fluorescence of released fluorophore can be measured and it is directly proportional to enzyme activity, because the fluorescence is measured at the end of the reaction the assay is referred to as an end-point assay.

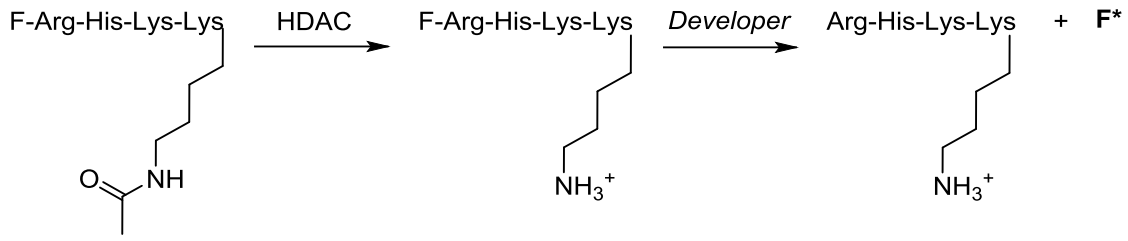


***Figure 5.1-1**: Illustration of the Fluor-de-lys assay kit. The fluorophore (F*) is excited with 380 nm light and the emitted light (460 nm) is detected on a fluorometric plate reader.*

SAHA and mocetinostat were tested against HDAC11 at 6 concentrations each. The assay of SAHA gave a rough IC_{50} of ~50 nM, which is lower than literature values (albeit closer than the value from Scottish Biomedical), but more importantly the plot of percentage inhibition against $\log_{10}$(concentration) had a good sigmoidal shape. The

assay of mocetinostat did not give a correctly shaped sigmoidal plot; it showed ~25% inhibition at 1 nM yet only ~75% inhibition at 100 µM. The literature report of mocetinostat's inhibition of HDAC11 was based on a similar assay which uses a slightly difference fluorescent substrate, MAL (Boc-Lys(Ac)-AMC).[68] The second developer step in this assay contains a commercially available enzyme (trypsin) to cleave the fluorophore from the deacetylated substrate ML (**Figure 5.1-2**).

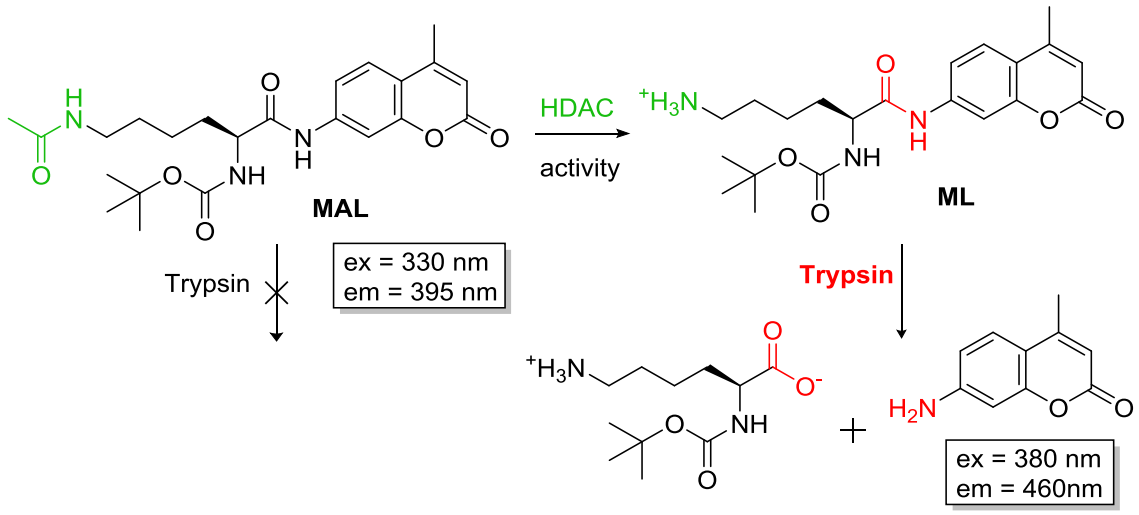
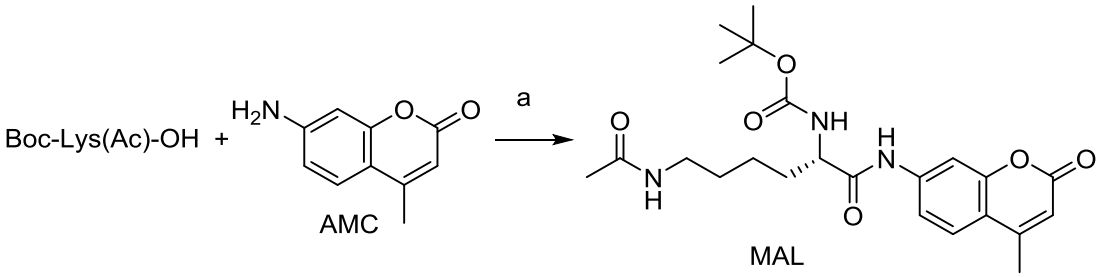
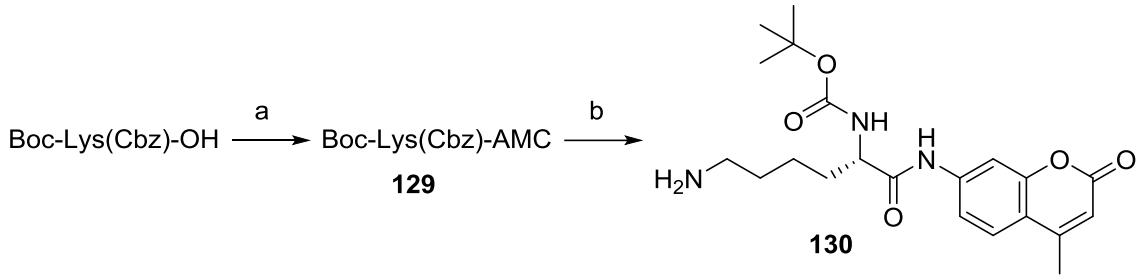


***Figure 5.1-2**: Illustration of the HDAC assay with MAL and trypsin.*

In order to reproduce as closely as possible the assay from the literature, MAL was synthesised (Scheme 5.1-1)[85], as was the deacetylated standard **130** which is needed to convert the fluorescence values from relative fluorescence units (RFU) to moles of substrate deacetylated, and **130** can also be used to check against false positive hits in the assay. If a compound inhibits trypsin, or if it absorbs radiation at the excitation or emission wavelength (380/460 nm), then it would appear to inhibit the HDAC enzyme in the assay. If the compound still appears to inhibit even when using the deacetylated standard **130** instead of using MAL, then it is a false positive.



***Scheme 5.1-2**: a) $POCl_3$, anh. pyridine, -10 °C, 30 min, 37%.*



***Scheme 5.1-3**: a) 7-amino-4-methylcoumarin, anh. pyridine, $POCl_3$, -10 °C, 1 h, 37%; b) H_2 balloon, 10% Pd/C, MeOH, rt, 5 h, 42%.*

The experimental details describing mocetinostat's IC_{50} for HDAC11 in the literature did not include the concentration of substrate used. Using the correct substrate concentration is vital for achieving a successful assay and an ideal concentration to use is in the range of the substrate's Michaelis constant (K_m) for that enzyme. Therefore a preliminary experiment was carried out using different concentrations of the substrate MAL and different reaction times in order to calculate the rate of reaction at different concentrations of MAL with a constant HDAC11 concentration (see **Figure 5.1-4** for a representative graph against HDAC3-NCoR1). Then using the various reciprocal plots of the rate and substrate concentration data (see **Figure 5.1-5** for a representative graph with HDAC3-NCoR1), an approximate K_m value was determined to be 40 μM for HDAC11 and MAL. Subsequently this value was used as the concentration of substrate in the assay, and mocetinostat was tested against HDAC11 in an assay with MAL. However again the plot of $\log_{10}$(concentration) against response was not a good fit to the expected sigmoidal curve. It was then reasoned that there could be some problem with the HDAC11 enzyme that was purchased, however Zhou *et al.* did not detail the source of their HDAC11. As the most recently identified HDAC, few inhibition data are available for this isoform and few commercial sources of the enzyme exist.

In order to check whether the problem was with the enzyme and not with another aspect of assay, compounds were tested against another isoform. HDAC8 was chosen since it was readily available in large quantities (thanks to Simon Greenwood for providing HDAC8) because it can be expressed in *Escherichia coli* BL21 cells and the active protein successfully purified with a His-tag which was removed prior to final purification by size exclusion chromatography. Although it was expected that the compounds prepared in this project would be less potent against HDAC8, plots that fitted the expected sigmoidal curve would show that the assay was functioning correctly. The K_m of MAL for HDAC8 was roughly calculated as being >1 mM (in

agreement with a literature value of >500 μM),[88] this calculation was limited by the solubility of MAL, and so for use in assays 200 μM was used (higher concentrations gave solubility problems). The assays with HDAC8 were successful, although as expected all benzamide compounds prepared had an IC_{50} value >1 μM (see tables in section 3.3.1) the graph below in **Figure 5.1-3** shows an example sigmoidal curve of mocetinostat against HDAC8, the points fit the curve well and the curve has limits at 0 and 100% inhibition.

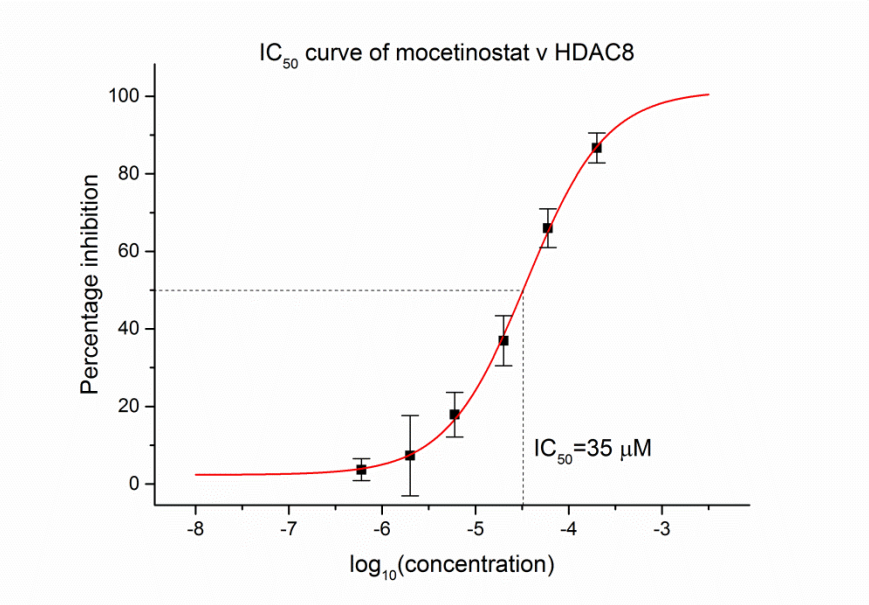


***Figure 5.1-3**: Graph showing sigmoidal curve of percentage inhibition against log_{10}(concentration) for mocetinostat against HDAC8. Points are the mean value from duplicate experiments and error bars are standard deviation from the mean.*

The other HDAC enzymes available from commercial sources are produced in insect cells (Sf9) because when made in *E. coli* cells they cannot be successfully purified or are enzymatically inactive. It is thought that there are post-translational modifications crucial for enzymatic activity that cannot occur in *E. coli* cells since these cells lack the necessary modifying enzymes. However HDAC8 does not have a flexible *C*-terminal tail known to contain some of the sites of post-translational modifications in other isoforms. Since HDAC11 is the smallest HDAC, it also lacks a *C*-terminal tail and it shares highest sequence homology with HDAC8 (see **Figure 1.2-3**, p 12) it was thought that expression of HDAC11 in *E. coli* might provide some active HDAC11 for use in assays. Several attempts were made to express and purify HDAC11 from *E. coli* cells using a similar expression system to that used for HDAC8; however HDAC11 protein was not successfully obtained from these efforts.

It was therefore decided to focus on the other isoforms that mocetinostat

inhibits: HDAC1, 2 and 3. These isoforms were also available commercially, and so they were purchased from Enzo Life Sciences, HDAC3 was supplied as a fusion protein with the deacetylase activation domain of the nuclear receptor co-repressor 1 complex (HDAC3-NCoR1). The K_m values of MAL against HDAC2 and HDAC3-NCoR1 were roughly calculated as ~80 and ~14 μM respectively using the procedure described above for HDAC11 (see representative graphs below), but for HDAC1 a K_m value was found in the literature (58.9 μM)[136].

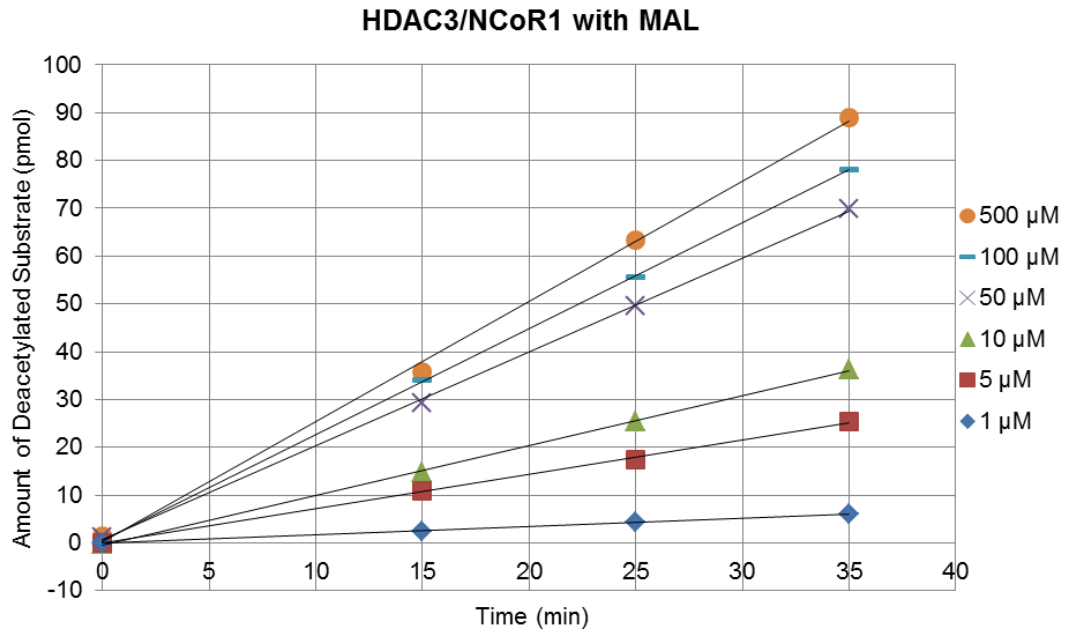


***Figure 5.1-4**: Graph showing amount of deacetylated substrate at different time points for HDAC3-NCoR1 with different concentrations of MAL substrate.*

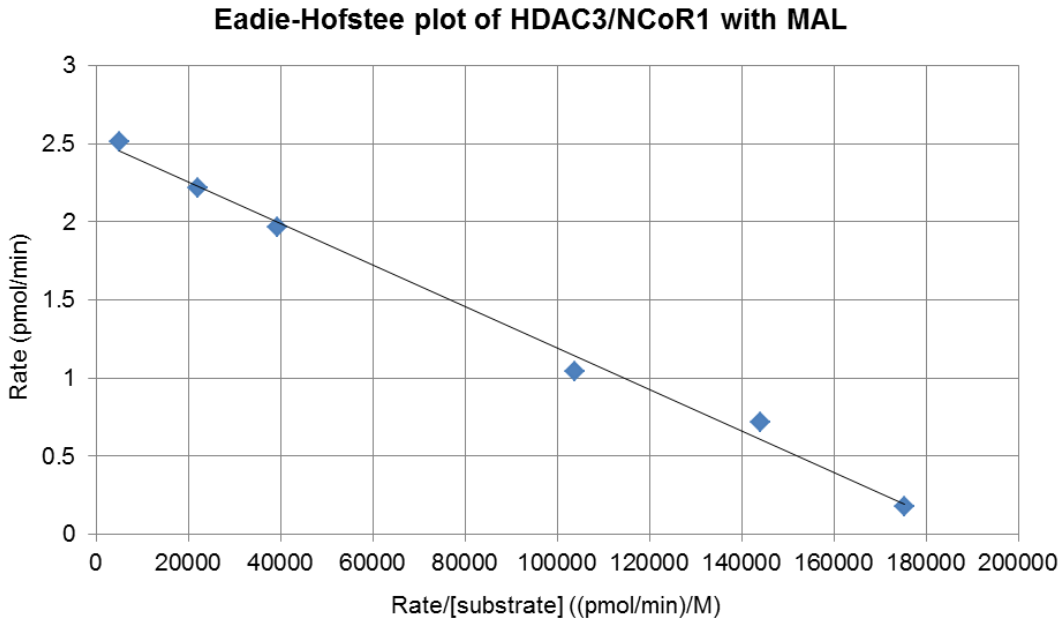


***Figure 5.1-5**: Graph of rate against rate/substrate concentration. Km = gradient × −1.*

A selection of benzamide HDAC inhibitors have been shown to be slow binding for certain HDAC enzymes (see section 1.4.2, p 29). Therefore when assessing the inhibitory activity of these benzamide inhibitors in an end-point assay, the length of pre-incubation of enzyme and inhibitor is crucial. An experiment was carried out with mocetinostat, against each isoform, comparing different lengths of pre-incubation. It was found that for HDAC3-NCoR1 even after 3 h pre-incubation the IC_{50} value had not reached equilibrium (**Figure 5.1-6**). However the longer the enzyme is left at room temperature, the more degradation and loss of activity occurs thereby reducing the resolution of the assay. So for HDAC3-NCoR1 three hours was decided as optimum, for HDAC1, 1 h was found to be optimum and for HDAC2, 3 h. The same process had been carried out for HDAC8 before testing against that isoform and pre-incubation made little difference in that case, so 15 min pre-incubation was used for the HDAC8 assays.

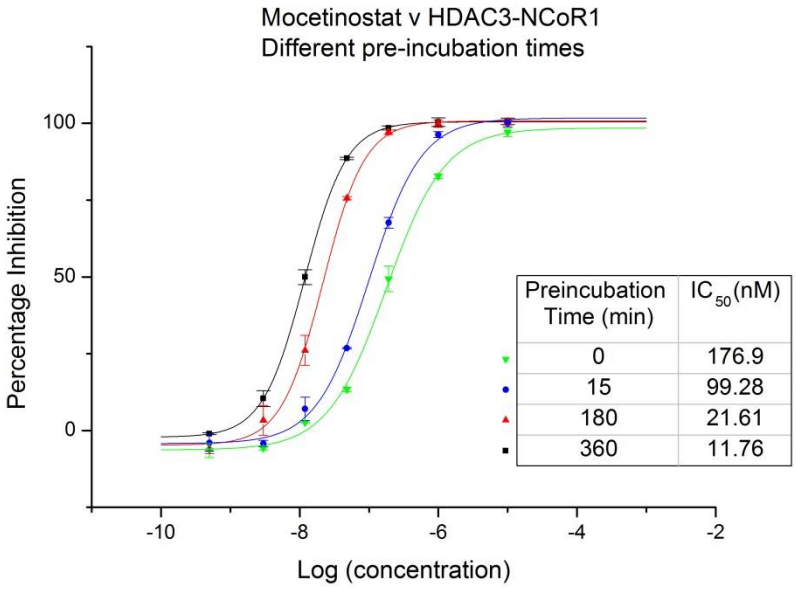


***Figure 5.1-6**: Graph showing percentage inhibition against log_{10}(concentration) for mocetinostat against HDAC3-NCoR1 with different pre-incubation times. Data points are mean values from duplicate experiments and error bars are SD from mean. Inset table shows IC_{50} values (nM) for each pre-incubation time.*

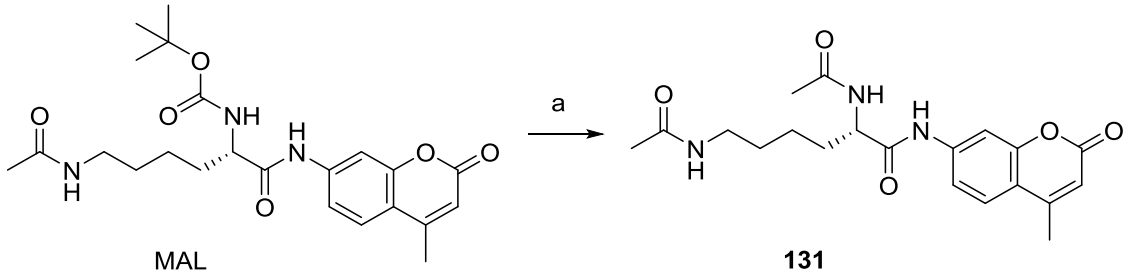
As the project progressed and more assays were carried out, new batches of HDAC enzymes were purchased. These new batches often behaved very differently in the assays to the original batches, sometimes having different activities and in some cases the inhibition of the new batch (by the same compounds) differed significantly. This can be seen for mocetinostat against HDAC6 comparing **Table 3.3-4** with **Table 3.3-5**, the percentage inhibition against the first batch of HDAC6 was <45% at 20 µM but against the second batch of enzyme, inhibition at the same concentration was

significantly higher (68%), these were the same HDAC6 kits both from Enzo Life Sciences, ordered approximately 18 months aparrt. Enzymes made by BPS Bioscience, US and distributed in the UK by TebuBio were found to be more active and consistent than those produced by Enzo Life Sciences.

5.2 Progression method assay

The endpoint assay discussed in the previous section, is a good assay for inhibitors that have fast on/off kinetics such as hydroxamic acids, however some benzamide HDAC inhibitors are known to be slow tight-binding inhibitors for certain isoforms (as discussed in section 1.4.2, p 29). The effect of introducing a longer pre-incubation time in the assay partially accounts for the slow on rate, however the assay is non-optimal for evaluating slow on/off inhibitors. For this reason an alternative assay was investigated; the progression method assay described by Chou, Herman and Gottesfeld.[67]

The assay follows the same basic principal as the end-point assay described in the previous section. Whereas that assay was carried out in two steps and a final fluorescence reading measured, the progression method assay contains both steps in 'one pot' and measures the fluorescence in real time over the course of the reaction. One primary difference that enables both steps to be carried out in 'one pot' is the use of a more selective enzyme, endoproteinase Lys-C, which cleaves the fluorophore from the deacetylated intermediate. Lys-C selectively hydrolyses the amide bond on the *C*-terminal side of a lysine residue, whereas trypsin which was used in the end-point assay above, cleaves on the *C*-terminal side of both lysine and arginine residues (except when followed by proline). The end result is that by using Lys-C in 'one pot' with the deacetylation reaction, the HDAC protein is degraded less quickly than it would be if trypsin were used. This means that the rate of a zero inhibitor control reaction is linear for enough time for the experiment to be useful, typically 45-60 mins. The assay also uses a slightly different substrate, Ac-Lys(Ac)-AMC (**131**) instead of MAL (Boc-Lys(Ac)-AMC), and therefore the new substrate was synthesised. Starting from MAL, the Boc-protecting group was removed, and then the resulting amine was acetylated using acetic anhydride, to afford **131** in 59% yield. The assay also requires the substrate concentration to be greater than the K_m, and according to the details in the literature **131** was used at 50 μM with HDAC3-NCoR1 (5 times the K_m).



***Scheme 5.2-1**: a) i) HCl in Et_2O, DCM, rt, 2.5 h;*
ii) acetic anhydride, DIPEA, MeCN, -10 °C, 2 h, 59%.

The fluorescence is measured every minute, and it is directly proportional to the amount of product produced in the assay. A non-linear regression of the integrated rate equation for slow binding inhibitors [$F = v_s t + (v_0 - v_s)(1 - \exp(-k_{obs}t))/k_{obs}$] is fit to the data to give a graph such as in **Figure 5.2-1** below.

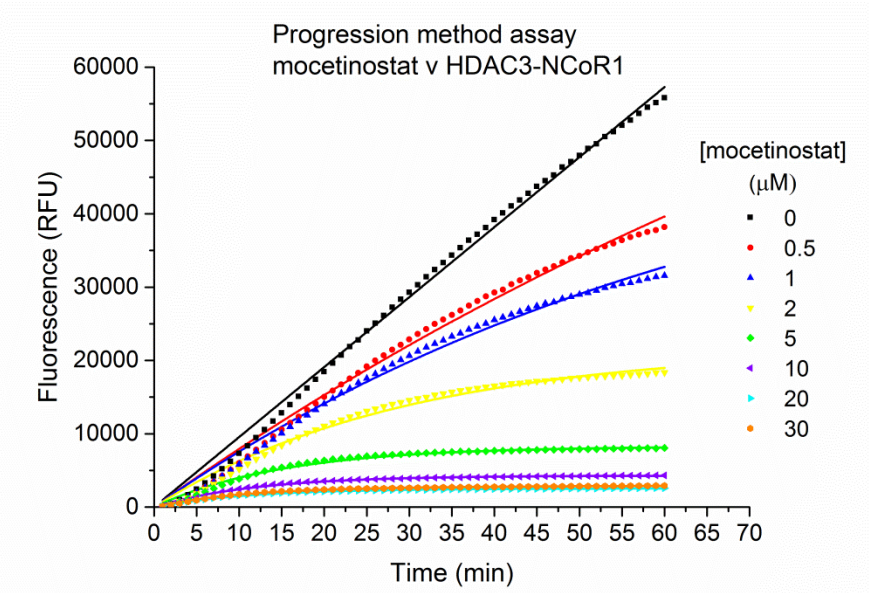


***Figure 5.2-1**: Graph showing the time dependent inhibition of HDAC3-NCoR1 as a function of mocetinostat concentration.*

From this non-linear regression, an observed rate constant k_{obs} can be calculated at each concentration of inhibitor. Subsequently a plot of these calculated k_{obs} values against the inhibitor concentration (**Figure 5.2-2**) gives information on the mechanism of slow binding (for more details see Chou *et al.*[67]).

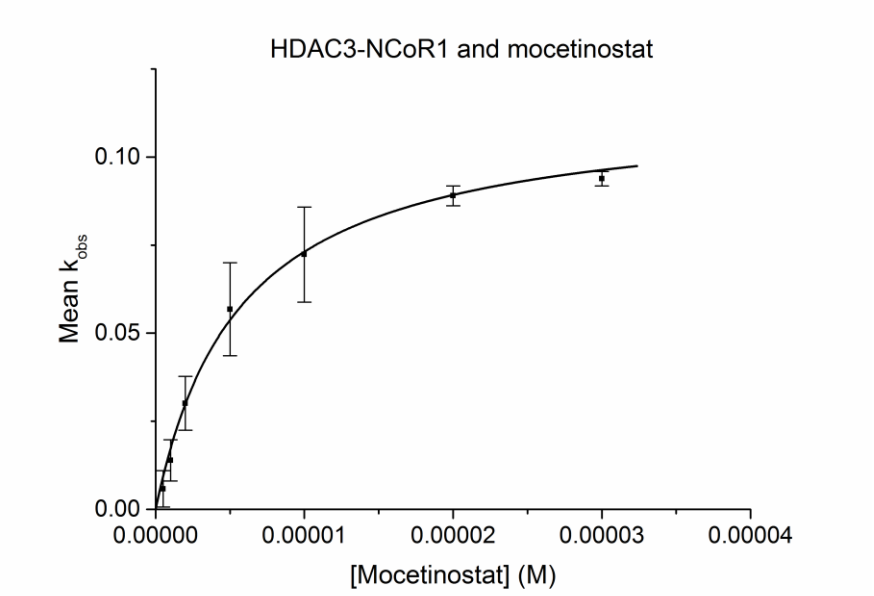


***Figure 5.2-2**: Graph showing hyperbolic relationship of k_{obs} and mocetinostat concentration against HDAC3-NCoR1. The mean k_{obs} value is from three independent experiments and error bars are standard deviation from the mean.*

As can be seen from the above graphs this assay was carried out with mocetinostat and HDAC3-NCoR1, and according to the hyperbolic shape of the graph in **Figure 5.2-2**, the slow binding mechanism is following a two-step process where the first initial fast binding of inhibitor and enzyme is followed by a slow second step such as a conformation change. According to Chou *et al.* the relationship between k_{obs} and inhibitor concentration for this mechanism is described by the following equation:

$$k_{obs} = k_{-2} + \frac{k_2[I]}{[I]} + K_i^*(1 + \frac{[S]}{K_m})$$

Fitting this equation to the data can reveal the inhibition constant K_i and on and off rate constants for the slow step of binding. However using OriginPro to fit this equation to the data collected for mocetinostat against HDAC3-NCoR1, resulted in numbers being produced such as $K_i=3.6 \times 10^{-21}$, which is many orders of magnitude different from the K_i that can be calculated from the end-point assays even after considering a longer pre-incuabtion time.

The same problem was found whilst carrying out the assay against HDAC2; whilst the plots fit the correct general shape the final numbers calculated are not sensible. Again a hyperbolic relationship between k_{obs} and mocetinostat concentration was observed and that indicates that again a two-step mechanism of inhibition is responsible for the slow binding. This is in contrast to the findings of Chou *et al.*, because their benzamide inhibitor was reported as having a single step mechanism for

HDAC2 (but a two-step mechanism for HDAC3).

Amino-linked thiazoline **(*S*)-59b** was also evaluated against HDAC2 and HDAC3-NCoR1 using this assay. Similarly to mocetinostat, **(*S*)-59b** demonstrates a two-step slow binding mechanism for both isoforms, however the final numbers generated (K_i, k_{on}, and k_{off}) are many orders of magnitude different to the results from the end-point assay.

In order to verify that the benzamide zinc-binding group is responsible for the slow tight-binding kinetics, hydroxamic acid analogue of mocetinostat **20** was also evaluated against HDAC3-NCoR1. In this case, as expected the rate is independent of time, because inhibition is independent of time on the timescale of minutes, therefore the graph consists of straight lines (**Figure 5.2-3**).

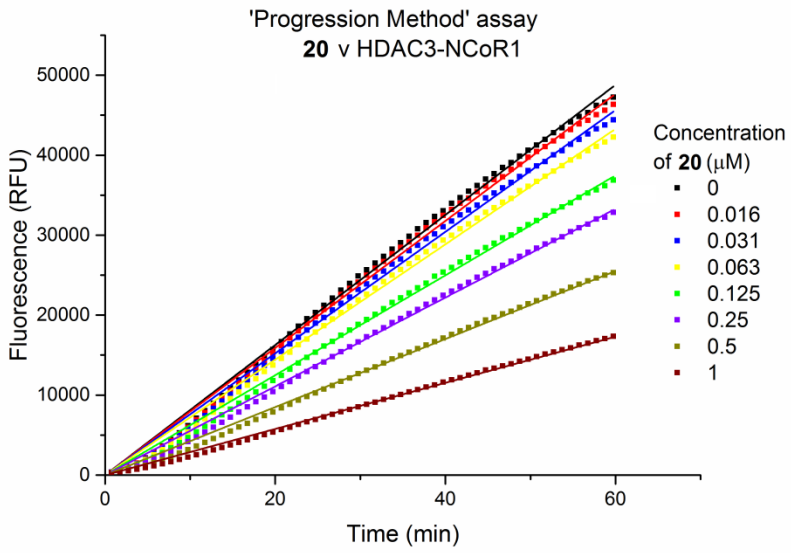


***Figure 5.2-3**: Graph showing the time independent inhibition of HDAC3-NCoR1 as a function of **20** concentration.*

This progression method assay was in theory better for evaluating slow tight-binding inhibitors, and it was able to provide additional information about the binding events such as on and off rates for the slow step. However in practice when using it to calculate quantitative data such as K_i, the calculated values were many orders of magnitude different from the K_i calculated using the more widely recognised end point assay. This could be because of an intrinsic problem with the assay explained below.

The fluorescence being measured in the assay is directly proportional to the amount of product from the second enzyme reaction. That second enzyme reaction is release of AMC from Ac-Lys-AMC, and at the start of the reaction the concentration of Ac-Lys-AMC (the substrate for the second step) is zero. During the first few minutes

the concentration of Ac-Lys-AMC increases which also means that the rate of the second step increases from zero at the start of the reaction. This means that the amount of product produced in the second reaction does not increase linearly at the start of the reaction, causing a lag period. Furthermore, since the length of lag period is dependent on the rate of production of Ac-Lys-AMC, it is different for different inhibitor concentrations and so it is not possible to easily adjust the equation for the fitting in order to account for this lag.

Mathematically an equation that incorporates this lag period can be derived (for the reaction without inhibitor) by looking at the equations which govern the rates of the reactions.

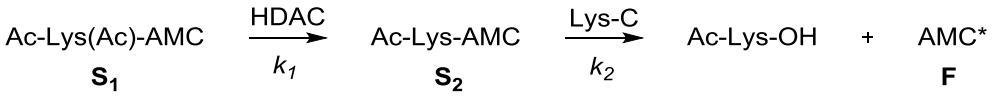


Figure 5.2-4: Progression method assay, showing abbreviated names used in the following equations below.

The rate of production of fluorescence (F) is governed by the following equation:

$$\frac{d[\mathrm{F}]}{dt} = k_2[\mathrm{S}_2] \qquad \textit{eq. 1}$$

An expression for the concentration of S_2 at a given time (t) can be derived by integrating the equation governing its rate of change and then rearranging:

$$\frac{d[S_2]}{dt} = k_1[\mathrm{S}_1] - k_2[\mathrm{S}_2] \qquad \textit{eq. 2}$$

$$[S_2] = k_1[\mathrm{S}_1]t - k_2[\mathrm{S}_2]t \qquad \textit{eq. 3}$$

$$[\mathrm{S}_2] = \frac{k_1[\mathrm{S}_1]t}{(1+k_2t)} \qquad \textit{eq. 4}$$

Substituting *eq. 4* into *eq. 1* and integrating gives the expected equation that a plot of fluorescence against time should fit (for the reaction without inhibitor).

$$\frac{d[\mathrm{F}]}{dt} = k_2\frac{k_1[\mathrm{S}_1]t}{(1+k_2t)} \qquad \textit{eq. 5}$$

$$\frac{d[\mathrm{F}]}{dt} = k_1[\mathrm{S}_1] - \frac{k_1[\mathrm{S}_1]}{(1+k_2t)} \qquad \textit{eq. 6}$$

$$[F] = k_1[\mathrm{S}_1]\left(t - \frac{\ln(k_2t+1)}{k_2}\right) \qquad \textit{eq. 7}$$

A plot of this equation roughly matches the observed shape of the reaction without inhibitor until the point that loss of activity can be seen because of enzyme degradation; an example plot of *eq. 7* is shown below.

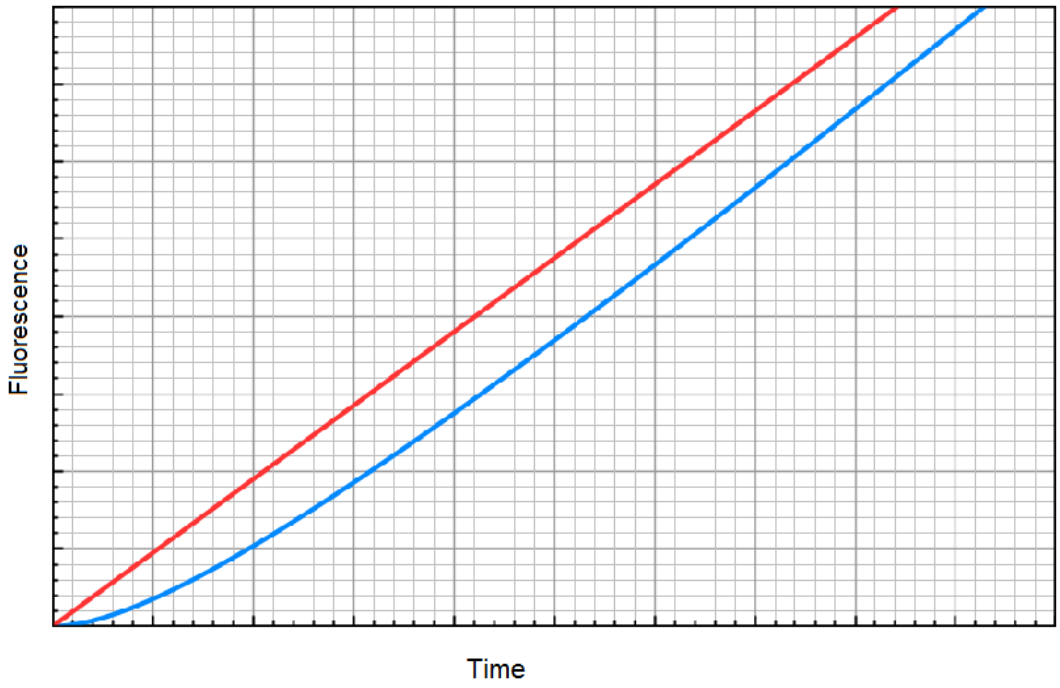


***Figure 5.2-5**: Example plot of eq. 7 (blue) and a straight line (red) to highlight the observed lag. Prepared using www.graphsketch.com.*

Another problem with this assay is that it is not applicable to all HDAC isoforms because of their sensitivity towards Lys-C degradation. Attempts were made to develop the assay for use with HDAC8; however this was not possible owing to the rapid loss of HDAC8 activity in the presence of Lys-C, even when using lower Lys-C concentrations than used with HDAC2/3.

The problems associated with this progression method assay meant that it was not used extensively for testing compounds because the end point assay was more useful for providing reliable quantitative data.

5.3 Conclusion

Several problems were encountered during the *in vitro* testing of HDAC inhibitors, especially obtaining IC_{50} values that are consistent with literature values. In fact self-consistency was also sometimes a difficulty because of the variation between different batches of the purchased enzymes. Certain HDAC enzymes are known to function as homo- and hetero-dimers and this could be part of the reason for the observed differences in inhibition. Another reason could be the non-trivial, slow tight-binding mechanism of benzamide HDAC inhibitors which make them highly sensitive to the conditions of the assay. These factors can also explain the large differences in

reported literature values for some benzamide HDAC inhibitors. For this reason an alternative type of *in vitro* assay was optimised to measure inhibition as a function of time, to account for the slow tight-binding nature of the inhibitors, however this assay also has its own limitations when attempting to use it to generate IC_{50} values. Whilst not perfect, the combination of these assays is at least able to give a rank order of inhibitor potency, determine isoform selectivity for the compounds prepared and demonstrate the time dependent nature of inhibition.

6 Conclusion

In conclusion the project has been partially successful, the aims of the project were not fully achieved, but progress was made towards synthesising isoform selective HDAC inhibitors.

The primary focus of the project was to develop inhibitors that are selective for inhibiting HDAC11; however this was not possible because of difficulties in setting up or outsourcing a reliable and reproducible fluorescent assay against HDAC11. Therefore there is some uncertainty about literature values for the inhibition of HDAC11 by mocetinostat and other inhibitors. The biological role of HDAC11 and the precise potential for HDAC11 selective inhibitors to treat various different diseases remains undetermined.

Assays with other HDAC isoforms were far more consistent and IC_{50} values reported in the literature were largely reproduced. However it is worth noting that reproducibility of assay results was sometimes not as high as desired when testing compounds with different enzyme batches, or enzymes from different suppliers. It is also important to consider the exact set-up and protocol that is followed, especially in view of the slow-tight binding nature of certain benzamide HDAC inhibitors.

It is important to reflect on the current options for *in vitro* testing of HDAC inhibitors, and the relevance of these assays when considering the use of inhibitors in an *in vivo* setting. Since the HDAC enzymes exist and function in multi-protein complexes, the inhibition of these complexes is likely to be different from the inhibition of the isolated purified recombinant proteins. Small changes to the isoform selectivity profile upon moving to an *in vivo* setting could change greatly the pharmacological result. It would be beneficial to the field if more work was done to establish robust *ex vivo* based assay techniques using the enzymes in their native multi-protein complexes, which can be used at a moderately high throughput scale and these assays must be well validated using a variety of different zinc-binding group inhibitors. Furthermore because of the complication of kinetic effects of slow binding inhibitors, an assay that can directly measure kinetics of inhibition would also be advantageous. There are some HDAC assays using Microfluidic Mobility Shift Assay systems[95] and if these can incorporate HDAC enzymes in their native multi-protein complexes, yet provide information on isoform selectivity (perhaps using a proteomics based approach[96]) this would be of great benefit.

The different approaches to making isoform selective inhibitors had different levels of success. Attempts to find a new zinc-binding group with some inherent isoform selectivity were unsuccessful; however the potential number of zinc-binding fragments that could be prepared and tested is vast. So this is still an area which might prove successful in the future; L-cysteine and thiosalicylic acid are two fragments that could deserve some further attention based on the screening result of the fragment units; they showed some inhibition of HDAC8 but lower levels of inhibition against other isoforms.

Development of a highly convergent synthetic route to make benzamide HDAC inhibitors containing a chiral heterocyclic capping group allowed the preparation of a large number of HDAC inhibitors to probe the effect of chirality on isoform selectivity. This has been shown to be a promising approach, increasing isoform selectivity within class I HDAC enzymes.

The next steps for the chemistry of this work would be to further investigate different chiral heterocyclic rings, particularly different sized rings, since the orientation of the chiral substituent will have additional options. Likewise a more thorough study is needed into the effect that different capping substituents have on inhibition and selectivity. This may also allow the pharmacokinetic properties of the compounds to be adjusted.

7 Experimental

General Experimental

All chemicals were used as supplied, except phenylene-1,2-diamine which was recrystallized from ethanol prior to use. Column chromatography was carried out using (40-60 μm) silica gel and analytical TLC was carried out using Merck Keiselgel aluminium-backed plates coated with silica gel. Components were visualised using a combination of ultra-violet light and potassium permanganate. Melting points were determined using an Electrothermal melting point apparatus. Infrared (IR) spectra were recorded on a PerkinElmer spectrum 100 FT-IR spectrometer as neat powders or thin films. ^{1}H NMR spectra were recorded at 300 MHz on a Bruker AMX300 spectrometer, at 400 MHz on a Bruker AMX400, at 500 MHz on a Bruker Avance 500 spectrometer, or at 600 MHz on a Bruker Avance 600 spectrometer in the stated solvent using residual protic solvent chloroform-*d*, methanol-d_4, or DMSO-d_6 as the internal standard. Chemical shifts are quoted in ppm. 13C NMR spectra were at 125 MHz on a Bruker Avance 500 spectrometer or at 150 MHz on a Bruker Avance 600 spectrometer in the stated solvent using the central reference of chloroform-*d*, methanol-d_4, or DMSO-d_6 as the internal standard. Mass spectra were performed at the Department of Chemistry, University College London. Optical rotations were measured using a PerkinElmer 343 polarimeter.

7.1 Experimental Procedures

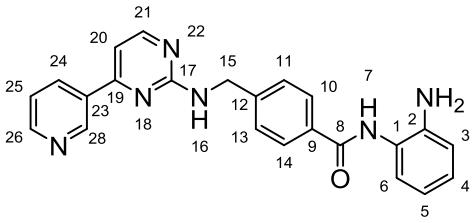
General procedure A (synthesis of thioureas):

To a solution of the amino alcohol (1 - 1.2 eq) in acetonitrile (4 mL/mmol) was added **22** (1 eq) and the resulting suspension was stirred at rt for 16 h, then evaporated.

General procedure B (cyclisation of thioureas into 2-aminothiazolines):

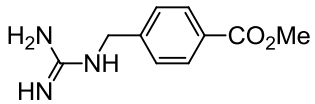
A solution of the thiourea (1 mmol) in conc. hydrochloric acid (20 mL, 10M) was heated at 90 °C under reflux with stirring for the given length of time. Aqueous sodium hydroxide (5M) was then added to pH ~ 9; and the mixture was extracted with ethyl acetate (3 × 50 mL). The combined organic layers were washed with brine (2 × 100 mL), dried Na_2SO_4, and evaporated.

_N_-(2-aminophenyl)-4-({[4-(pyridin-3-yl)pyrimidin-2-yl]amino}methyl)benzamide (mocetinostat)[97]



To a solution of **9** (1.46 g, 4.77 mmol, 1.0 eq), BOP (2.43 g, 5.48 mmol, 1.15 eq) and phenylene-1,2-diamine (1.03 g, 9.53 mmol, 2.0 eq) in DMF (48 ml) was added triethylamine (1.33 ml, 9.53 mmol, 2.0 eq) and then stirred at rt for 24 h. Most of the DMF was evaporated. The resulting dark orange oil was purified by column chromatography on silica gel (ethyl acetate to 5% methanol:ethyl acetate) to give an orange sticky solid which was triturated with dichloromethane and filtered to give mocetinostat as a white powder (1.12 g, 2.83 mmol, 59%), mp 168-169 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 4.65 (2H, d, *J*=3.5 Hz, CH_2), 4.87 (2H, br s, NH_2), 6.58 (1H, t, *J*=7.3 Hz, H(5)), 6.76 (1H, d, *J*=7.6 Hz, H(3)), 6.95 (1H, t, *J*=7.4 Hz, H(4)), 7.14 (1H, d, *J*=7.3 Hz, H(6)), 7.25 (1H, d, *J*=5.0 Hz, H(20)), 7.41 - 7.58 (3H, m, H(25), H(11 and 13)), 7.92 (2H, d, *J*=7.6 Hz, H(10 and 14)), 8.00 (1H, br t, *J*=5.2 Hz, $CH_2N\mathit{H}$), 8.37 - 8.43 (2H, m, H(24) and H(21)), 8.67 (1H, br s, H(26)), 9.24 (1H, br s, H(28)), 9.59 (1H, br s, CONH); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 44.0 (CH_2), 106.1 (C(20)), 116.1 (C(3)), 116.2 (C(5)), 123.4 (C(1)), 123.8 (C(25)), 126.4 (C(6)), 126.6 (C(4)), 126.9 (C(11 and 13)), 127.8 (C(10 and 14)), 132.4 (C(23)), 133.0 (C(9)), 134.2 (C(24)), 143.1 (C(2)), 144.1 (C(12)), 148.0 (C(28)), 151.3 (C(26)), 159.6 (C(19) and C(21)), 162.4 (C(17)), 165.2 (C=O).

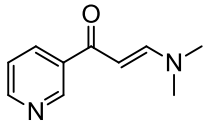
Methyl 4-(guanidinomethyl)benzoate (6)[137]



To a solution of methyl 4-(aminomethyl)benzoate hydrochloride (3.19 g, 15.8 mmol, 1.0 eq) in abs. ethanol (15 ml) was added 1*H*-pyrazole-1-carboxamidine hydrochloride (2.80 g, 19.1 mmol, 1.2 eq) and *N*,*N*-diisopropylethylamine (8.25 ml, 47.4 mmol, 3.0 eq). The mixture was stirred at reflux for 3 h. The solvent was evaporated and to the remaining oil was added 30 ml sat. aq. sodium hydrogen carbonate slowly under vigorous stirring followed by 190 ml water to achieve ~pH 9. A

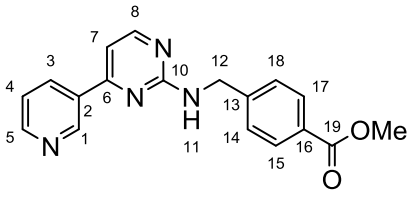
white solid was formed and stirring continued for 1 h. The white precipitate was collected by filtration and washed with water and diethyl ether then triturated with diethyl ether to give **6** as a fine white powder (2.61 g, 12.6 mmol, 80 %), mp 158-159 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 3.85 (3H, s, CO_2CH_3), 4.38 (2H, s, CH_2Ar), 7.40 (2H, d, *J*=8.3 Hz, ArH), 7.93 (2H, d, *J*=8.3 Hz, ArH); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 43.4 (CH_2), 52.1 (CH_3), 127.1 (3, 5-aryl), 128.4 (1-aryl), 129.3 (2, 6-aryl), 143.8 (4-aryl), 157.5 (C=N), 166.0 (C=O).

(*E*)-3-(Dimethylamino)-1-(pyridin-3-yl)prop-2-en-1-one (7)[97]



3-Acetylpyridine (1.96 ml, 17.8 mmol) was added to *N*,*N*-dimethylformamide dimethyl acetal (4.75 ml, 35.6 mmol) and the solution refluxed for 5 h. The reaction was then evaporated and the brown solid triturated with diethyl ether and filtered to give **7** as a yellow solid (2.62 g, 14.8 mmol, 83%), mp 84-85 °C (lit. 70-71)[138]; ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 2.94 (3H, s, CH_3), 3.17 (3H, s, CH_3), 5.67 (1H, d, *J*=12.0 Hz, COCH), 7.35 (1H, dd, *J*=7.8, 4.8 Hz, 5-pyridyl), 7.84 (1H, d, *J*=12.0 Hz, C*H*NMe_2), 8.18 (1H, dt, *J*=7.8, 1.9 Hz, 4-pyridyl), 8.66 (1H, dd, *J*=4.8, 1.8 Hz, 6-pyridyl), 9.08 (1H, d, *J*=1.8 Hz, 2-pyridyl); ^{13}C NMR (75 MHz, chloroform-*d*) δ ppm 37.4 (NCH_3), 45.2 (NCH_3), 91.8 (CO*C*H), 123.3 (5-pyridyl), 135.1 (4-pyridyl), 135.7 (3-pyridiyl), 148.9 (CHN), 151.4 (2-pyridyl), 154.7 (6-pyridyl), 186.3 (C=O).

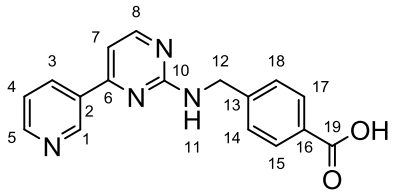
Methyl 4-((4-(pyridin-3-yl)pyrimidin-2-ylamino)methyl)benzoate (8)[97]



A suspension of (*E*)-3-(dimethylamino)-1-(pyridin-3-yl)prop-2-en-1-one (**7**) (1.85 g, 10.5 mmol, 1.0 eq) and methyl 4-(guanidinomethyl)benzoate (**6**) (2.5 g, 12.1 mmol, 1.15 eq) in propan-2-ol (25 ml) was stirred at reflux for 20 h. The orange solution was evaporated to dryness and the yellow residue triturated with cold ethyl acetate twice to give **8** as a cream powder (2.25 g, 67%), mp 149-50 °C. A portion was recrystallized from propan-2-ol to give a white solid, mp 153-154 °C; ^{1}H NMR (500

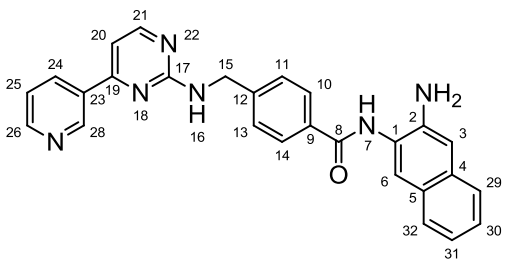
MHz, DMSO-d_6) δ ppm 3.80 (3H, s, OCH_3), 4.64 (2H, d, *J*=5.7 Hz, H(12)), 7.24 (1H, d, *J*=5.0 Hz, H(7)), 7.43 - 7.55 (3H, m, H(14 and 18) and H(3)), 7.90 (2H, d, *J*=8.2 Hz, H(10 and 14)), 7.98 (1H, t, *J*=6.1 Hz, H(4)), 8.32 - 8.41 (2H, m, H(8) and H(11)), 8.66 (1H, br s, H(5)), 9.19 (1H, br s, H(1)); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 44.0 (C(12)), 52.0 (OCH_3), 106.2 (C(7)), 123.8 (C(4)), 127.2 (C(14 and 18), 127.9 (C(16)), 129.2 (15 and 17), 132.3 (C(2)), 134.2 (C(3)), 146.4 (C(13)), 148.0 (C(1)), 151.3 (C(5)), 159.3 (C(6) and C(8)), 162.4 (C(10)), 166.1 (C(19)).

4-((4-(Pyridin-3-yl)pyrimidin-2-ylamino)methyl)benzoic acid (9)[97]



To a solution of methyl 4-((4-(pyridin-3-yl)pyrimidin-2-ylamino)methyl)benzoate (**8**) (1.60 g, 5.0 mmol) in THF (30 ml) was added a solution of lithium hydroxide monohydrate (0.525 g, 12.5 mmol, 2.5 eq) in water (15 ml) then stirred at room temperature for 24 h. THF was evaporated and then the resulting suspension was diluted with water (30 ml) and acidified with 1M aq. hydrochloric acid until pH 5-6, the precipitate was filtered and rinsed with water and dried to give **9** as a cream solid (1.49 g, 4.87 mmol, 97%), mp 210-211 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 4.66 (2H, d, *J*=5.8 Hz, H(12)), 7.26 (1H, d, *J*=5.0 Hz, H(7)), 7.44 - 7.58 (3H, m, H(14 and 18) and H(4)), 7.90 (2H, d, *J*=8.0 Hz, H(15 and 17)), 7.99 (1H, t, *J*=6.0 Hz, H(11)), 8.41 (2H, d, *J*=5.0 Hz, H(8) and H(3)), 8.69 (1H, br s, H(5)), 9.24 (1H, br s, H(1)), 12.85 (1H, br s, CO_2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 44.0 (C(12)), 106.2 (C(7)), 123.9 (C(4)), 127.1 (C(14 and 18)), 129.1 (C(16)), 129.3 (C(15 and 17)), 132.4 (C(2)), 134.2 (C(3)), 145.8 (C(13)), 147.9 (C(5)), 151.2 (C(1)), 159.3 (C(6) and C(8)), 162.4 (C(10)), 167.2 (C(19)).

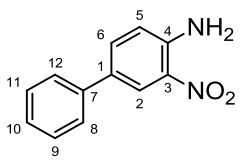
***N*-(3-aminonaphthalen-2-yl)-4-({[4-(pyridin-3-yl)pyrimidin-2-yl]amino}methyl)benzamide (10)**



Carboxylic acid **9** (0.238 g, 0.77 mmol) was stirred at reflux in $SOCl_2$ (1 ml) and toluene (3 ml) for 3 h. The solution was evaporated and azeotroped with toluene to give a white solid which was dissolved in dichloromethane (5 ml) and triethylamine (0.11 ml, 0.77 mmol).

Naphthalene-2,3-diamine (0.134 g, 0.847 mmol, 1.1 eq) was dissolved in dichloromethane (5 ml) and triethylamine (0.11 ml, 0.77 mmol) and DMF (0.2 ml). The solution was cooled to 0 °C and to this stirring cooled solution was added the above solution of acid chloride dropwise over 10 min. Stirred at 0 °C for a further 30 min and then allowed to warm to rt whilst stirring for 1 h. Then sat. aq. sodium hydrogen carbonate (10 ml) was added and diluted with water (10 ml) and dichloromethane (15 ml), the aqueous layer was extracted with dichloromethane (25 ml). The combined organics were washed with brine and dried Na_2SO_4 and evaporated. Purification by column chromatography (hexane to ethyl acetate) gave **10** as a tan solid (0.11 g, 0.246 mmol, 32%), mp 185-187 °C; ν_{max} (cm^{-1}) 3411 (NH), 3332 (NH), 3224 (NH), 1640 (C=O); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 4.67 (2H, d, *J*=6.0 Hz, CH_2N), 5.23 (2H, br s, NH_2), 7.08 (1H, s, H(3)), 7.13 (1H, ddd, *J*=8.2, 6.8, 1.0 Hz, H(31)), 7.25 (1H, d, *J*=5.2 Hz, H(20)), 7.27 (1H, ddd, *J*=8.4, 6.8, 1.1 Hz, H(30)), 7.46 - 7.57 (4H, m, H(11), H(13), H(25) and H(29)), 7.65 (1H, d, *J*=8.2 Hz, H(32)), 7.85 (1H, s, H(6)), 7.97 (2H, d, *J*=8.4 Hz, H(10) and H(14)), 7.99 (1H, t, *J*=6.0 Hz, CH_2NH), 8.38 - 8.42 (2H, m, H(24) and H(21)), 8.68 (1H, d, *J*=3.8 Hz, H(26)), 9.24 (1H, br s, H(28)), 9.72 (1H, s, CONH); ^{13}C NMR (150 MHz, DMSO-d_6) δ ppm 44.0 (CH_2), 106.1 (C(20)), 108.7 (C(3)), 121.7 (C(6)), 123.9 (C(25)), 124.1 (C(31)), 124.8 (C(30)), 125.5 (C(29)), 126.4 (C(1)), 126.5 (C(5)), 127.0 (C(11 and 13)), 127.2 (C(32)), 127.9 (C(10 and 14)), 132.4 (C(23)), 132.8 (C(4)), 133.0 (C(9)), 134.2 (C(24)), 142.2 (C(2)), 144.4 (C(12)), 148.1 (C(28)), 151.3 (C(26)), 159.2 (C(21), 159.7 (C(19)), 162.4 (C(17)), 165.8 (C=O); *m/z* (ES^+) 469 ($[M+Na]^+$, 50%), 307 ($[M-(C_6H_4NH_2)+3H]^{2+}$, 100%); HRMS $C_{27}H_{22}N_6ONa^+$ requires: 469.1753, found: 469.1774.

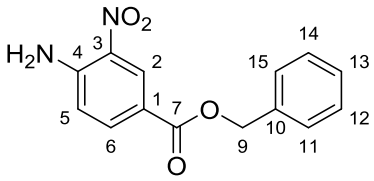
2-nitro-4-phenylaniline (11)[139]



To a solution of 4-bromo-2-nitroaniline (0.434 g, 2.0 mmol) and phenylboronic acid (0.293 g, 2.4 mmol, 1.2 eq) in 1,2-dimethoxyethane (17 ml) was added sodium

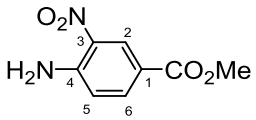
carbonate (2M aq., 2.1 ml, 4.0 mmol, 2 eq) and $Pd(PPh_3)_4$ (0.116 g, 0.1 mmol, 0.05 eq). The reaction mixture was stirred at 80 °C for 48 h (reaction may have been complete sooner but the product and bromo-aniline starting materials had exactly the same r.f. by TLC in a number of different solvent systems). After cooling, diluted with ethyl acetate (10 ml) and water (10 ml), aq. layer was extracted with ethyl acetate (20 ml), and the combined organics were washed with 10% sodium hydroxide (15 ml) and brine, dried Na_2SO_4 and evaporated to give an orange solid. Purification by column chromatography on silica gel (hexane:ethyl acetate, 3:1) gave an orange crystalline solid that was recrystallized from ethanol and water to give **11** as an orange crystalline solid (0.239 g, 1.12 mmol, 56%), mp 169-170 °C (lit.[140] 169-171 °C); ν_{max} (cm^{-1}) 3476 (NH), 3344 (NH), 1639 (NO); ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 6.15 (2H, br s, NH_2), 6.92 (1H, d, J=8.8 Hz, H(5)), 7.36 (1H, t, J=7.3 Hz, H(10)), 7.46 (2H, t, J=7.5 Hz, H(9) and H(11)), 7.58 (2H, d, J=7.3 Hz, H(8) and H(12)), 7.67 (1H, dd, J=8.7, 2.1 Hz, H(6)), 8.40 (1H, d, J=2.0 Hz, H(2)); 13C NMR (125 MHz, chloroform-*d*) δ ppm 119.4 (C(5)), 124.0 (C(2)), 126.4 (C(8 and 12)), 127.4 (C(10)), 129.1 (C(9 and 11)), 130.5 (C(1)), 132.5 (C(3)), 134.6 (C(6)), 138.9 (C(7)), 143.9 (C(4)).

benzyl 4-amino-3-nitrobenzoate (12)



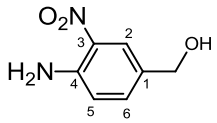
To a stirred solution of 4-amino-3-nitrobenzoic acid (0.728 g, 4.0 mmol, 1.0 eq) in anhydrous DMF (20 ml) was added potassium carbonate (0.608 g, 4.4 mmol, 1.1 eq) followed by dropwise addition of benzyl bromide (0.476 ml, 4.0 mmol, 1.0 eq). The mixture was stirred at rt for 18 h then poured onto water (125 ml) and extracted with ethyl acetate (3 × 75 ml), the combined organics were washed with brine (4 × 50 ml) then dried $MgSO_4$ and concentrated to give **12** as a yellow solid (1.04 g, 3.82 mmol, 96%), mp 125-127 °C; ν_{max} (cm^{-1}) 3472 (NH), 3343 (NH), 1692 (C=O); ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 5.37 (2H, s, C(9)H_2), 6.48 (2H, br s, NH_2), 6.85 (1H, d, *J*=8.8 Hz, H(5)), 7.33 - 7.52 (5H, m, ArH), 8.03 (1H, dd, *J*=8.8, 2.0 Hz, H(6)), 8.89 (1H, d, *J*=2.0 Hz, H(2)); ^{13}C NMR (125 MHz, chloroform-*d*) δ ppm 66.9 (C(9)), 118.6 (C(5)), 119.1 (C(1)), 128.4 (C(11 and 15)), 128.5 (C(2)), 128.7 (C(12 and 14)), 129.2 (C(13)), 131.6 (C(3)), 135.9 (C(10)), 136.0 (C(6)), 147.5 (C(4)), 164.9 (C(7)); *m/z* (EI^+) 272 (M^+, 100%); HRMS $C_{14}H_{12}N_2O_4^+$ requires: 272.0792, found: 272.0803.

methyl 4-amino-3-nitrobenzoate (13)[141]



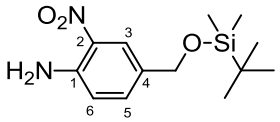
4-amino-3-nitrobenzoic acid (1.82 g, 10.0 mmol) suspended in methanol (20 ml) then cooled to 0 °C and $SOCl_2$ (0.726 ml, 10.0 mmol) was added dropwise. After addition was complete stirred at reflux for 16 h, then cooled to 0 °C and filtered washed with cold methanol. The solid was dried under vacuum to give **13** as a yellow crystalline solid (1.84 g, 9.37 mmol, 94%), mp 199-200 °C (lit.[142] 198 °C); ν_{max} (cm^{-1}) 3473 (NH), 3338 (NH), 1698 (C=O), 1627 (NO); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 3.85 (3H, s, CH_3), 7.04 - 7.15 (1H, m, H(6)), 7.89 (1H, d, *J*=6.9 Hz, H(5)), 8.00 (2H, br s, NH_2), 8.58 (1H, s, H(2)); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 52.0 (CH_3), 116.2 (C(1)), 119.4 (C(5)), 128.1 (C(2)), 129.7 (C(3)), 134.9 (C(6)), 148.9 (C(4)), 165.0 (C=O).

(4-amino-3-nitrophenyl)methanol (14)



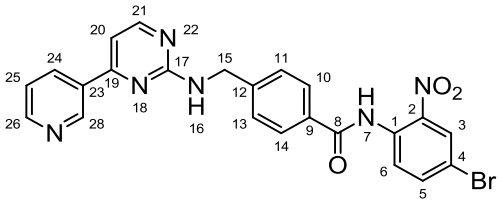
Methyl ester **13** (0.20 g, 1.02 mmol) and a stirrer bar were charged to a flask, the flask was then flushed with nitrogen, anhydrous THF (7 ml) was added, and the solution cooled to 0 °C. To this solution was added DIBAL (1M in dichloromethane, 2.6 ml, 2.6 mmol, 2.5 eq) dropwise over 10 min keeping temperature below 5 °C. Stirred in ice bath for 1 h, then allowed to warm to rt and stirred for a further 3 h. Cooled to 0 °C and then methanol (3 ml) was added dropwise, followed by sat. aq. K/Na tartrate (10 ml) and ethyl acetate (20 ml). Stirred at rt for 2 h then separated and the aqueous layer extracted with ethyl acetate (50 ml and 25 ml). The combined organic layers were washed with brine, dried $MgSO_4$ and concentrated to give **13** as a crystalline orange solid (0.169 g, 1.0 mmol, 98%) mp 102-104 °C (lit.[143] 100-101 °C). A small amount was recrystallized from ethanol and hexane to give a dark orange crystalline solid, mp 106-107 °C; ν_{max} (cm^{-1}) 3437 (NH), 3328 (NH), 1641 (NO); ^{1}H NMR (400 MHz, methanol-d_4) δ ppm 4.50 (2H, s, CH_2), 6.96 (1H, d, *J*=8.8 Hz, H(5)), 7.38 (1H, dd, *J*=8.8, 2.0 Hz, H(6)), 8.04 (1H, d, *J*=2.0 Hz, H(2)); ^{13}C NMR (125 MHz, methanol-d_4) δ ppm 64.1 (CH_2O), 120.3 (C(5)), 124.9 (C(2)), 130.6 (C(1)), 132.0 (C(3)), 136.2 (C(6)), 146.8 (C(4)).

4-{[(*tert*-butyldimethylsilyl)oxy]methyl}-2-nitroaniline (15)



The alcohol **14** (0.5 g, 3.0 mmol, 1.0 eq), imidazole (0.612 g, 9.0 mmol, 3.0 eq) and *tert*-butyldimethylsilyl chloride (0.678 g, 4.5 mmol, 1.5 eq) were dissolved in anhydrous DMF (1.5 ml) and stirred at rt under a nitrogen balloon for 16 h. The solution was then diluted with water (40 ml) and extracted with diethyl ether (2 × 30 ml). The combined organic layers were washed with 5% aq. lithium chloride (3 × 20 ml) and brine (20 ml) then dried Na_2SO_4 and evaporated. Purification by column chromatography on silica gel (dichloromethane) gave **15** as a yellow crystalline solid (0.755 g, 2.67 mmol, 89%), mp 69-70 °C; ν_{max} (cm^{-1}) 3490 (NH), 3372 (NH), 1638 (C=O); ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 0.12 (6H, s, 2 × $SiCH_3$), 0.95 (9H, s, $C(CH_3)_3$), 4.64 (2H, s, CH_2O), 6.07 (2H, br s, NH_2), 6.82 (1H, d, *J*=8.5 Hz, H(6)), 7.37 (1H, dd, *J*=8.5, 2.0 Hz, H(5)), 8.07 (1H, d, *J*=2.0 Hz, H(3)); ^{13}C NMR (125 MHz, chloroform-*d*) δ ppm -5.1 (2 × $SiCH_3$), 18.4 ($SiC(CH_3)_3$), 26.0 ($C(CH_3)_3$), 64.0 (CH_2O), 119.0 (C(6)), 123.5 (C(3)), 130.6 (C(4)), 131.9 (C(2)), 134.4 (C(5)), 143.9 (C(1)); *m/z* (EI^+) 282 (M^+, 7%), 225 ($[M\text{-}C(CH_3)_3]^+$, 100 %), 207 ($[M\text{-}C(CH_3)_3\text{-water}]^+$, 67%); HRMS $C_{13}H_{22}N_2O_3Si^+$ requires: 282.1394, found: 282.1403.

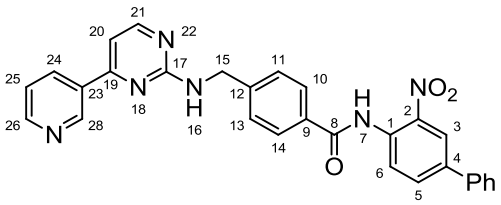
***N*-(4-bromo-2-nitrophenyl)-4-({[4-(pyridin-3-yl)pyrimidin-2-yl]amino}methyl)benzamide (16a)**



To a solution of 4-bromo-2-nitroaniline (0.217 g, 1.00 mmol, 1.0 eq), carboxylic acid **9** (0.367 g, 1.20 mmol, 1.2 eq) and BOP (0.522 g, 1.18 mmol, 1.18 eq) in pyridine (7 ml) was added sodium hydride (60% in mineral oil, 0.268 g, 6.7 mmol, 6.7 eq) portionwise under a stream of nitrogen. Stirred at rt for 2.5 h, then diluted with toluene (3 ml) and quenched with acetic acid (1 ml). The solvent was evaporated and then azeotroped with toluene. Dissolved in ethyl acetate (150 ml) and washed with sat. aq. sodium hydrogen carbonate (200 ml). The aqueous layer was extracted with ethyl acetate (100 ml), and the combined organics were washed with water (50 ml) and brine

(100 ml), then dried $MgSO_4$ and evaporated to give an orange/yellow solid. Purification by column chromatography on silica gel (ethyl acetate) and recrystallization from acetic acid and water gave **16a** as a yellow solid (0.354 g, 0.700 mmol, 70%), mp 197-198 °C; ν_{max} (cm^{-1}) 3351 (NH), 3248 (NH), 1689 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 4.67 (2H, d, *J*=6.3 Hz, H(15)), 7.27 (1H, d, *J*=5.3 Hz, H(20)), 7.47 - 7.63 (3H, m, H(25) and H(11 and 13)), 7.72 (1H, d, *J*=8.8 Hz, H(6)), 7.90 (2H, d, *J*=8.3 Hz, H(10 and 14)), 7.95 (1H, dd, *J*=8.7, 2.1 Hz, H(5)), 8.04 (1H, t, *J*=6.3 Hz, H(16)), 8.20 (1H, d, *J*=2.3 Hz, H(3)), 8.34 - 8.48 (2H, m, H(21) and H(24)), 8.69 (1H, br s, H(26)), 9.24 (1H, br s, H(28)), 10.76 (1H, s, H(7)); ^{13}C NMR (150 MHz, DMSO-d_6) δ ppm 44.0 (CH_2), 106.2 (C(20)), 116.5 (C(4)), 123.9 (C(25)), 127.3 (C(10 and 14)), 127.5 (C(6)), 127.6 (C(3)), 127.9 (C(11 and 13)), 131.0 (C(9)), 131.5 (C(1)), 132.4 (C(23)), 134.2 (C(24)), 136.7 (C(5)), 143.3 (C(2)), 145.5 (C(12)), 148.1 (C(26)), 151.3 (C(28)), 159.7 (C(19)), 161.0 (C(21)), 162.4 (C(17)), 165.2 (C=O); *m/z* (ES^+) 505 (M^+, 98%); HRMS $C_{23}H_{18}N_6O_3Br^+$ requires: 505.0624, found: 505.0604.

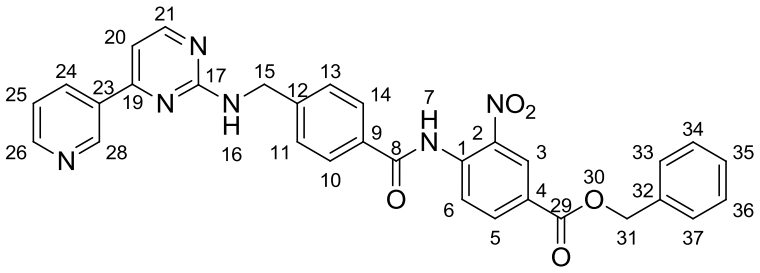
N-(2-nitro-4-phenylphenyl)-4-({[4-(pyridin-3-yl)pyrimidin-2-yl]amino}methyl)benzamide (16b)



To a solution of 4-phenyl-2-nitroaniline **11** (0.215 g, 1.00 mmol, 1.0 eq), carboxylic acid **9** (0.361 g, 1.18 mmol, 1.18 eq) and BOP (0.522 g, 1.18 mmol, 1.18 eq) in pyridine (7 ml) was added sodium hydride (60% in mineral oil, 0.268 g, 6.7 mmol, 6.7 eq) portionwise under a stream of nitrogen. Stirred at rt for 5 h then BOP (0.050 g, 0.0113 mmol, 0.113 eq) was added and stirred at rt for 1 h, then sodium hydride (60% in mineral oil, 0.026 g, 0.67 mmol, 0.67 eq) added stirred at rt for 1 h. The mixture was diluted with toluene (2 ml) and quenched with acetic acid (2 ml). The solvent was evaporated and then azeotroped with toluene. Dissolved in ethyl acetate (150 ml) and washed with sat. aq. sodium hydrogen carbonate (100 ml). The aqueous layer was extracted with ethyl acetate (100 ml), and the combined organics were washed with brine (2 × 50 ml), then dried Na_2SO_4 and evaporated to give an orange/yellow solid. Purification by column chromatography on silica gel (hexane:ethyl acetate, 1:1 to 1:3) and recrystallization from acetic acid followed by stirring in sat. aq. sodium carbonate for 16 h then filtration gave **16b** as a yellow solid (0.194 g, 0.387 mmol, 39%), mp 208-

209 °C; ν_{max} (cm^{-1}) 3349 (NH), 3264 (NH), 1693 (C=O), 1675 (NO); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 4.67 (2H, d, *J*=5.8 Hz, CH_2), 7.26 (1H, d, *J*=5.0 Hz, H(20)), 7.42 (1H, t, *J*=7.3 Hz, 4-phenyl), 7.51 (5H, m, 3,5-phenyl, H(25), H(11) and H(13)), 7.76 (2H, d, *J*=7.6 Hz, 2,6-phenyl), 7.87 (1H, d, *J*=8.2 Hz, H(5)), 7.92 (2H, d, *J*=7.9 Hz, H(10) and H(14)), 8.00 (1H, t, *J*=5.8 Hz, CH_2NH), 8.06 (1H, d, *J*=8.2 Hz, H(6)), 8.23 (1H, s, H(3)), 8.35 - 8.46 (2H, H(24) and H(21)), 8.67 (1H, br s, H(26)), 9.23 (1H, br s, H(28)), 10.73 (1H, br s, CONH); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 44.0 (CH_2), 106.3 (C(20)), 122.6 (6)), 123.8 (C(25)), 126.2 (C(3)), 126.7 (C(10 and 14)), 127.3 (C(11 and 13)), 127.8 (2,6-phenyl), 128.3 (C(1)), 129.2 (3,5-phenyl), 130.8 (C(9)), 131.8 (C(5)), 131.8 (4-phenyl), 132.4 (C(23)), 134.2 (C(24)), 137.1 (C(4)), 137.5 (1-phenyl), 143.0 (C(2)), 145.3 (C(12)), 148.0 (C(26)), 151.3 (C(28)), 159.5 (C(19) and C(21)), 162.4 (C(17)), 165.2 (C=O); *m/z* (ES^+) 503 ($[M+H]^+$, 100%); HRMS $C_{29}H_{23}N_6O_3^+$ requires: 503.1832, found: 503.1818.

benzyl 3-nitro-4-{[4-({[4-(pyridin-3-yl)pyrimidin-2-yl]amino}methyl)benzene]amido}benzoate (16c)

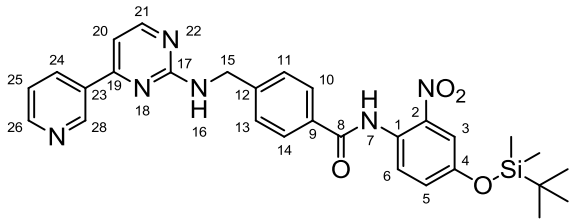


A suspension of benzoic acid **9** (0.452 g, 1.48 mmol, 1.2 eq) in $SOCl_2$ (2 ml) and toluene (2 ml) was stirred at reflux for 3 h then evaporated to give the acid chloride as a cream solid.

In a 2-neck oven-dried flask **12** (0.335 g, 1.23 mmol, 1.0 eq) was dissolved in anhydrous pyridine (5 ml) and the solution cooled to -10 °C (salt-ice bath) under a nitrogen balloon. To this solution was added 60% sodium hydride in mineral oil (0.148 g, 3.69 mmol, 3.0 eq) and then stirred at -10 °C for 5 min (the yellow solution became a deep red colour). To this red solution at -10 °C was added the acid chloride prepared above portionwise as a cream solid under a nitrogen balloon over 5 min. Stirred at rt for 2 h then quenched with acetic acid (2 ml), diluted with toluene (5 ml) and evaporated. The resulting oil was co-evaporated with toluene and then purified by column chromatography on silica gel (ethyl acetate) to give **16c** as a pale yellow solid (0.353 g, 0.629 mmol, 51%), mp 175-176 °C; ν_{max} (cm^{-1}) 3343 (NH), 3215 (NH), 1720 (C=O),

1683 (C=O); ^{1}H NMR (600 MHz, DMSO-d_6) δ ppm 4.67 (2H, br s, C(15)H_2), 5.39 (2H, s, C(31)H_2), 7.27 (1H, d, *J*=4.9 Hz, H(20)), 7.37 (1H, t, *J*=7.2 Hz, H(35)), 7.42 (2H, t, *J*=7.2 Hz, H(34 and 36)), 7.50 (2H, d, *J*=7.2 Hz, H(33 and 37)), 7.51 - 7.62 (3H, m, H(25) and H(11 and 13)), 7.92 (2H, d, *J*=8.3 Hz, H(10 and 14)), 8.02 (1H, d, *J*=8.5 Hz, H(6)), 8.04 (1H, t, *J*=6.2 Hz, H(16)), 8.29 (1H, dd, *J*=8.5, 1.7 Hz, H(5)), 8.35 - 8.45 (2H, m, H(21) and H(24)), 8.48 (1H, d, *J*=1.7 Hz, H(3)), 8.68 (1H, br s, H(26)), 9.23 (1H, br s, H(28)), 11.02 (1H, s, H(7)); ^{13}C NMR (150 MHz, DMSO-d_6) δ ppm 44.0 (C(15)), 66.8 (C(31)), 106.2 (C(20)), 123.9 (C(25)), 125.3 (C(6)), 125.6 (C(4)), 126.1 (C(3)), 127.3 (C(10 and 14)), 128.0 (C(11 and 13)), 128.2 (C(33 and 37)), 128.3 (C(35)), 128.6 (C(34 and 36)), 131.4 (C(9)), 132.4 (C(23)), 134.2 (C(24)), 134.4 (C(5)), 135.8 (C(2)), 136.1 (C(32)), 141.5 (C(1)), 145.8 (C(12)), 148.1 (C(26)), 151.3 (C(28)), 159.2 (C(19)), 159.6 (C(21)), 162.4 (C(17)), 163.7 (C(29)), 165.3 (C(8)); *m/z* (ES^+) 561 ($[M+H]^+$, 100%); HRMS $C_{31}H_{25}N_6O_5^+$ requires: 561.1886, found: 561.1877.

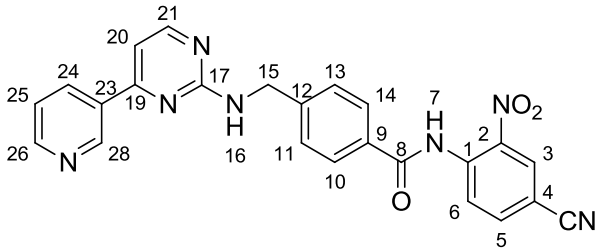
_N_-(4-{[(*tert*-butyldimethylsilyl)oxy]methyl}-2-nitrophenyl)-4-({[4-(pyridin-3-yl)pyrimidin-2-yl]amino}methyl)benzamide (16d)



To a solution of arylamine **15** (0.700 g, 2.48 mmol, 1.2 eq), carboxylic acid **9** (0.634 g, 2.07 mmol, 1.0 eq) and BOP (0.916 g, 2.07 mmol, 1.0 eq) in pyridine (15 ml) was added sodium hydride (60% in mineral oil, 0.580 g, 14.5 mmol, 7.0 eq) portionwise under a stream of nitrogen. Stirred at rt for 2.5 h, then the mixture was diluted with toluene (7 ml) and quenched with acetic acid (4 ml). The solvent was evaporated and then azeotroped with toluene. Dissolved in ethyl acetate (250 ml) and washed with water (150 ml), sat. aq. sodium hydrogen carbonate (150 ml) and brine (100 ml), then dried Na_2SO_4 and evaporated to give an orange/yellow solid. Purification by column chromatography on silica gel (hexane:ethyl acetate, 1:3) gave **16d** as a yellow solid (0.490 g, 0.859 mmol, 41%), mp 144-146 °C; ν_{max} (cm^{-1}) 3356 (NH), 3228 (NH), 1684 (C=O); ^{1}H NMR (500 MHz, chloroform-*d*) δ ppm 0.12 (6H, s, 2 × $SiCH_3$), 0.95 (9H, s, $C(CH_3)_3$), 4.76 (2H, s, CH_2O), 4.82 (2H, d, *J*=6.1 Hz, CH_2N), 5.97 (1H, br s, CH_2NH), 7.04 (1H, d, *J*=5.2 Hz, H(20)), 7.39 (1H, dd, *J*=7.9, 4.7 Hz, H(25)), 7.55 (2H, d, *J*=8.4 Hz, H(11) and H(13)), 7.63 (1H, dd, *J*=8.8, 2.0 Hz, H(5)), 7.96 (2H, d, *J*=8.4 Hz, H(10)

and H(14)), 8.24 (1H, d, *J*=2.0 Hz, H(3)), 8.27 (1H, dt, *J*=7.9, 1.9 Hz, H(24)), 8.38 (1H, d, *J*=5.0 Hz, H(21)), 8.68 (1H, dd, *J*=4.7, 1.4 Hz, H(26)), 8.94 (1H, d, *J*=8.8 Hz, H(6)), 9.19 (1H, d, *J*=1.6 Hz, H(28)), 11.29 (1H, s, CONH); ^{13}C NMR (150 MHz, chloroform-*d*) δ ppm -5.2 (2 × $SiCH_3$), 18.5 (Si*C*$(CH_3)_3$), 26.0 (C(*C*$H_3)_3$), 45.2 (CH_2N), 63.6 (CH_2O), 107.1 (C(20)), 122.2 (C(6)), 123.1 (C(3)), 123.7 (C(25)), 127.8 (C(10 and 14)), 127.9 (C(11 and 13)), 132.9 (C(23)), 133.0 (C(9)), 133.9 (C(5)), 134.1 (C(1)), 134.6 (C(24)), 136.4 (C(2)), 137.4 (C(4)), 144.7 (C(12)), 148.6 (C(28)), 151.4 (C(26)), 159.2 (C(21)), 162.5 (C(19)), 162.6 (C(17)), 165.6 (C=O); *m/z* (ES^+) 571 ($[M+H]^+$, 100%); HRMS $C_{30}H_{35}N_6O_4Si^+$ requires: 571.2489, found: 571.2483.

***N*-(4-cyano-2-nitrophenyl)-4-({[4-(pyridin-3-yl)pyrimidin-2-yl]amino}methyl)benzamide (16e)**

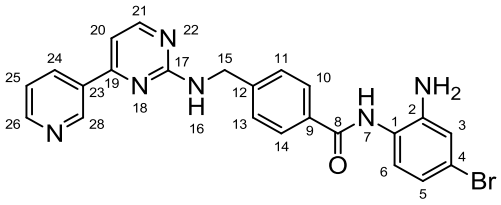


A suspension of benzoic acid **9** (0.225 g, 0.734 mmol, 1.0 eq) in $SOCl_2$ (1 ml) and toluene (1 ml) was stirred at reflux for 2 h then evaporated and co-evaporated with toluene to give the acid chloride as a cream solid.

In a 2-neck oven-dried flask 4-amino-3-nitro--benzonitrile (0.120 g, 0.734 mmol, 1.0 eq) was dissolved in anhydrous pyridine (2 ml) and the solution cooled to -10 °C (salt-ice bath) under a nitrogen balloon. To this solution was added 60% sodium hydride in mineral oil (88 mg, 2.20 mmol, 3.0 eq) and then stirred at -10 °C for 10 min (the yellow solution became a deep red colour). To this red solution at -10 °C was added the acid chloride prepared above portionwise as a cream solid under an nitrogen balloon over 10 min. Stirred at 0°C for 1 h then a further portion of 60% sodium hydride in mineral oil (44 mg, 1.10 mmol, 1.5 eq) was added and stirred at 0°C to rt for 4 h. Then quenched with acetic acid (1 ml), diluted with toluene (3 ml) and evaporated. The resulting oil was co-evaporated with toluene and then purified by column chromatography on silica gel (ethyl acetate) to give **16e** as a white solid (0.146 g, 0.324 mmol, 44%), mp 183-184 °C; ν_{max} (cm^{-1}) 3349 (NH), 3238 (NH), 1695 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 4.68 (2H, d, *J*=6.3 Hz, C(15)H_2), 7.28 (1H, d, *J*=5.0 Hz, H(20)), 7.47 - 7.65 (3H, m, (H(11 and 13) and H25), 7.91 (2H, d, *J*=8.3 Hz, H(10 and 14)), 7.99 (1H, d, *J*=8.5 Hz, H(6)), 8.04 (1H, t, *J*=6.3 Hz, H(16)), 8.19 (1H, dd, *J*=8.5,

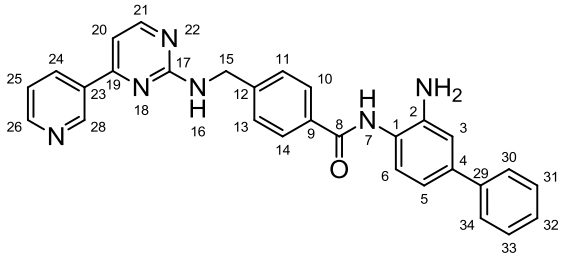
2.0 Hz, H(5)), 8.36 - 8.46 (2H, m, H(21) and H(24)), 8.57 (1H, d, *J*=2.0 Hz, H(3)), 8.69 (1H, d, *J*=3.5 Hz, H(26)), 9.24 (1H, br s, H(28)), 11.05 (1H, br s, H(7)); ^{13}C NMR (150 MHz, DMSO-d_6) δ ppm 44.0 (C(15)), 106.2 (C(20)), 107.1 (C≡N), 117.2 (C(4)), 123.9 (C(25)), 125.7 (C(6)), 127.3 (C(10 and 14)), 128.0 (C(11 and 13)), 129.7 (C(3)), 131.2 (C(9)), 132.4 (C(23)), 134.2 (C(24)), 135.8 (C(2)), 137.3 (C(5)), 141.8 (C(1)), 145.9 (C(12)), 148.1 (C(26)), 151.3 (C(28)), 159.2 (C(19)), 159.6 (C(21)), 162.4 (C(17)), 165.4 (C(8)); *m/z* (ES^+) 452 ($[M+H]^+$, 71%); HRMS $C_{24}H_{18}N_7O_3^+$ requires: 452.1471, found: 452.1454.

_N_-(2-amino-4-bromophenyl)-4-({[4-(pyridin-3-yl)pyrimidin-2-yl]amino}methyl)benzamide (17a)



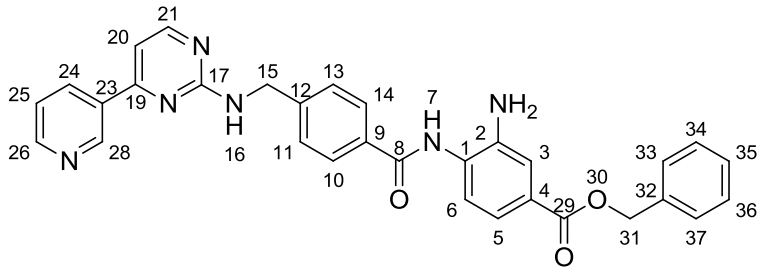
To a solution of $FeSO_4$.7water (1.87 g, 6.74 mmol, 12 eq) in water (4 ml) and 30% aq. ammonia (4 ml) heated to 60 °C was added a suspension of the nitro compound **16a** (0.28 g, 0.562 mmol, 1 eq) in ethanol (4 ml). Stirred at 60 °C for 4 h then cooled to rt and filtered through celite washing with 10% methanol/dichloromethane (100 ml) and DM 100 ml). The combined filtrate was washed with water (100 ml) and brine (100 ml), then dried $MgSO_4$ and evaporated to give a pale yellow solid (0.24 g). Purified by column chromatography on silica gel (5% methanol/chloroform) to give **17a** as a white solid (0.164 g, 0.345 mmol, 61%), mp 206-208 °C; ν_{max} (cm^{-1}) 3247 (NH), 1633 (C=O); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 4.64 (2H, d, *J*=6.2 Hz, CH_2N), 5.19 (2H, s, NH_2), 6.69 (1H, dd, *J*=8.4, 2.0 Hz, H(5)), 6.93 (1H, d, *J*=2.0 Hz, H(3)), 7.09 (1H, d, *J*=8.4 Hz, H(6)), 7.24 (1H, d, *J*=5.2 Hz, H(20)), 7.44 - 7.55 (3H, m, H(11), H(13) and H(25)), 7.90 (2H, d, *J*=8.2 Hz, H(10) and H(14)), 7.97 (1H, t, *J*=6.2 Hz, CH_2N*H*), 8.40 (2H, d, *J*=5.2 Hz, H(24) and H(21)), 8.67 (1H, d, *J*=3.6 Hz, H(26)), 9.23 (1H, br s, H(28)), 9.53 (1H, s, CONH); ^{13}C NMR (150 MHz, DMSO-d_6) δ ppm 44.0 (CH_2), 106.0 (C(20)), 117.7 (C(3)), 118.3 (C(5)), 118.8 (C(4)), 122.5 (C(1)), 123.9 (C(25)), 126.9 (C(11 and 13)), 127.9 (C(10 and 14)), 128.6 (C(6)), 132.4 (C(23)), 132.8 (C(9)), 134.2 (C(24)), 144.3 (C(12)), 145.1 (C(2)), 148.1 (C(28)), 151.3 (C(26)), 159.1 (C(21)), 159.6 (C(19)), 162.4 (C(17)), 165.4 (C=O); *m/z* (ES^+) 475 ($[M+H]^+$, 53%), 307 ($[M-(C_6H_3BrNH_2)+3H]^{2+}$, 100%); HRMS $C_{23}H_{20}N_6OBr^+$ requires: 475.0882, found: 475.0866.

_N_-(2-amino-4-phenylphenyl)-4-({[4-(pyridin-3-yl)pyrimidin-2-yl]amino}methyl)benzamide (17b)



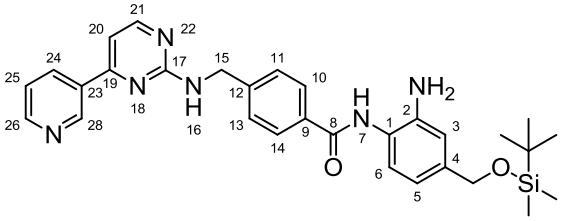
To a stirring suspension of **16b** (0.557 g, 1.11 mmol) in methanol (10 ml) and dichloromethane (10 ml) was added 10% palladium on activated carbon (0.118 g). The flask was then evacuated and filled with nitrogen three times to remove oxygen, finally the flask was evacuated and then filled with hydrogen gas from a balloon. The mixture was stirred vigorously under a hydrogen balloon for 6 h, then filtered through a thin layer of celite and washed with methanol (100 ml) and a 1:1 mixture of methanol and dichloromethane (100 ml) the combined filtrates were evaporated. Purification by column chromatography on silica gel (4% methanol/chloroform) gave **17b** as a white solid (0.165 g, 0.349 mmol, 31%), mp 207-209 °C; ν_{max} (cm^{-1}) 3338 (NH), 3239 (NH), 1635 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 4.67 (2H, d, *J*=5.3 Hz, CH_2), 5.04 (2H, br s, NH_2), 6.90 (1H, dd, *J*=8.0, 1.8 Hz, H(5)), 7.09 (1H, d, *J*=1.8 Hz, H(3)), 7.25 - 7.30 (2H, m, H(20) and H(6)), 7.33 (1H, t, *J*=7.0 Hz, H(32)), 7.44 (2H, t, *J*=7.7 Hz, H(31 and 33), 7.48 - 7.61 (5H, m, H(11 and 13), H(30 and 34) and H(25)), 7.96 (2H, d, *J*=8.0 Hz, H(10 and 14)), 8.05 (1H, t, *J*=6.3 Hz, H(16)), 8.43 (2H, d, *J*=5.3 Hz, H(24) and H(21)), 8.69 (1H, d, *J*=3.8 Hz, H(26)), 9.26 (1H, br s, H(28)), 9.66 (1H, s, H(7)); ^{13}C NMR (150 MHz, DMSO-d_6) δ ppm 44.0 (C(15)), 106.1 (C(20)), 114.2 (C(3)), 114.8 (C(5)), 123.0 (C(1)), 123.9 (C(25)), 126.4 (C(30 and 34)), 127.0 (C(11 and 13) and C(6)), 127.1 (C(32)), 127.9 (C(10 and 14)), 128.9 (C(31 and 33)), 132.4 (C(23)), 133.0 (C(9)), 134.2 (C(24)), 138.3 (C(29)), 140.5 (C(4)), 143.3 (C(2)), 144.3 (C(12)), 148.1 (C(28)), 151.3 (C(26)), 159.2 (C(19)), 159.7 (C(21)), 162.4 (C(17)), 165.3 (C(8)); *m/z* (ES^+) 473 ($[M+H]^+$, 47%), 307 ($[M+H\text{-water}]^+$, 48%); HRMS $C_{29}H_{25}N_6O^+$ requires: 473.2090, found: 473.2092.

benzyl 3-amino-4-[4-({[4-(pyridin-3-yl)pyrimidin-2-yl]amino}methyl)benzamido]benzoate (17c)



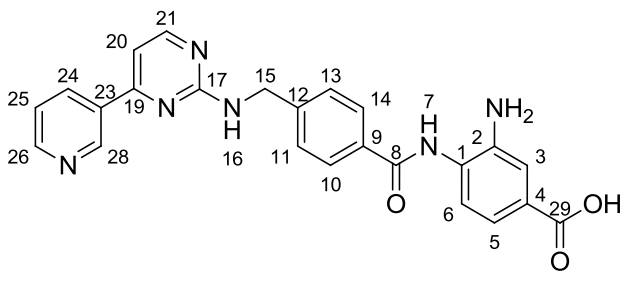
To a suspension of **16c** (147 mg, 0.262 mmol, 1.0 eq) in methanol (6 ml) and dichloromethane (4 ml) was added 10% palladium on activated carbon (28 mg). The flask was then evacuated and filled with nitrogen three times to remove oxygen, finally the flask was evacuated and then filled with hydrogen gas from a balloon. The mixture was stirred vigorously under a hydrogen balloon for 5.5 h, then filtered through a thin layer of celite and washed with methanol (3 × 20 ml) the combined filtrates were evaporated. Purification by column chromatography on silica gel (ethyl acetate) gave **17c** as a cream solid (25 mg, 0.0577 mmol, 22%), mp 149-151 °C; ν_{max} (cm^{-1}) 3431 (NH), 3213 (NH), 1712 (C=O), 1631 (C=O); ^{1}H NMR (600 MHz, DMSO-d_6) δ ppm 3.59 (2H, br s, NH_2 (and H_2O), 4.66 (2H, br s, C(16)H_2), 5.32 (2H, s, C(31)H_2), 7.22 - 7.32 (2H, m, H(20) and H(5)), 7.36 (1H, t, *J*=7.2 Hz, H(35)), 7.39 - 7.56 (8H, m, H(3), H(6), H(11 and 13), H(33 and 37), H(34 and 36)), 7.62 (1H, br s, H(25)), 7.93 (2H, d, *J*=8.3 Hz, H(10 and 14)), 8.08 (1H, br s, H(16)), 8.43 (1H, br s, H(21)), 8.51 (1H, d, *J*=7.2 Hz, H(24)), 8.73 (1H, br s, H(26)), 9.27 (1H, br s, H(28)), 9.76 (1H, br s, H(7)); ^{13}C NMR (150 MHz, DMSO-d_6) δ ppm 44.0 (C(15)), 66.0 (C(31)), 106.2 (C(20)), 117.2 (C(3)), 118.0 (C(5)), 124.3 (C(25)), 125.9 (C(6)), 127.0 (C(11 and 13)), 128.0 (C(10 and 14)), 128.0 (C(33 and 37)), 128.1 (C(4) and C(35)), 128.5 (C(1)), 128.6 (C(34 and 36)), 132.7 (C(23)), 132.8 (C(9)), 135.4 (C(24)), 136.3 (C(32)), 141.6 (C(2)), 144.5 (C(12)), 147.1 (C(28)), 150.3 (C(26)), 159.3 (C(21)), 159.5 (C(19)), 162.2 (C(17)), 165.4 (C(8)), 165.7 (C(29)); *m/z* (ES^+) 531 ($[M+H]^+$, 63%), 513 ($[M+H\text{-water}]^+$, 45%); HRMS $C_{31}H_{27}N_6O_3^+$ requires: 531.2145, found: 531.2123.

***N*-(2-amino-4-{[(*tert*-butyldimethylsilyl)oxy]methyl}phenyl)-4-({[4-(pyridin-3-yl)pyrimidin-2-yl]amino}methyl)benzamide (17d)**



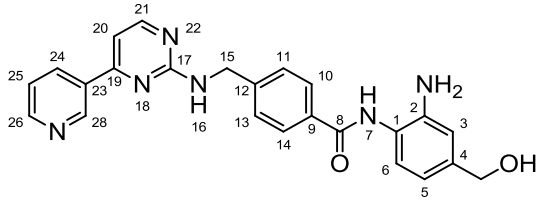
To a stirring suspension of **16d** (190 mg, 0.33 mmol) in methanol (8 ml) and chloroform (2 ml) was added 10% palladium on activated carbon (50 mg). The flask was then evacuated and filled with nitrogen three times to remove oxygen, finally the flask was evacuated and then filled with hydrogen gas from a balloon. The mixture was stirred vigorously under a hydrogen balloon for 7 h, then filtered through a thin layer of celite and washed with methanol (3 × 20 ml) the combined filtrates were evaporated. Purification by column chromatography on silica gel (ethyl acetate) gave **17d** as a tan solid (0.064 g, 0.118 mmol, 36%), mp 89-90 °C; ν_{max} (cm^{-1}) 3300 (NH), 1641 (C=O); ^{1}H NMR (400 MHz, methanol-*d*$_4$) δ ppm 0.12 (6H, s, 2 × $SiCH_3$), 0.96 (9H, s, $C(CH_3)_3$), 4.68 (2H, s, OCH_2), 4.77 (2H, s, NCH_2), 6.75 (1H, dd, J=8.0, 1.3 Hz, H(5)), 6.90 (1H, d, J=1.3 Hz, H(3)), 7.16 (1H, d, J=8.0 Hz, H(6)), 7.20 (1H, d, J=5.3 Hz, H(20)), 7.54 - 7.58 (3H, m, H(25), H(11) and H(13)), 7.96 (2H, d, J=8.3 Hz, H(10) and H(14)), 8.39 (1H, d, J=5.3 Hz, H(21)), 8.46 (1H, d, J=7.3 Hz, H(24)), 8.64 (1H, dd, J=4.8, 1.5 Hz, H(26)), 9.19 (1H, br s, H(28)); ^{13}C NMR (125 MHz, methanol-*d*$_4$) δ ppm -4.7 ($SiCH_3$), 19.4 (Si*C*$(CH_3)_3$), 26.7 (C(*C*$H_3)_3$), 45.9 (CH_2N), 66.1 (CH_2O), 107.6 (C(20)), 116.8 (C(5)), 118.1 (C(3)), 124.4 (C(1)), 125.4 (C(6)), 127.5 (C(25)), 128.5 (C(10 and 14)), 129.1 (C(11 and 13)), 134.1 (C(9)), 134.8 (C(23)), 136.6 (C(24)), 142.0 (C(4)), 143.0 (C(2)), 145.7 (C(12)), 149.0 (C(26)), 151.7 (C(28)), 160.2 (C(21)), 163.5 (C(19)), 163.8 (C(17)), 168.8 (C=O); *m/z* (ES^+) 541 ($[M+H]^+$, 100%), 523 ([M+H-water]$^+$, 38%); HRMS $C_{30}H_{37}N_6O_2Si^+$ requires: 541.2747, found: 541.2756.

3-amino-4-[4-({[4-(pyridin-3-yl)pyrimidin-2-yl]amino}methyl)benzamido]benzoic acid (17f)



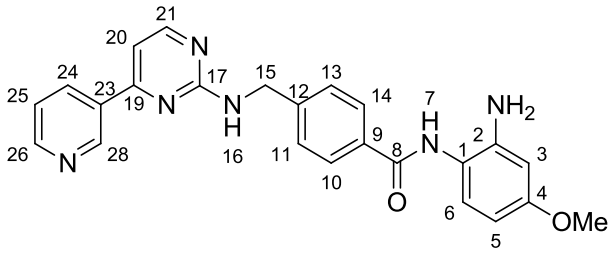
To a suspension of **16c** (320 mg, 0.571 mmol, 1.0 eq) in methanol (6 ml) was added 10% palladium on activated carbon (120 mg). The flask was then evacuated and filled with nitrogen three times to remove oxygen, finally the flask was evacuated and then filled with hydrogen gas from a balloon. The mixture was stirred vigorously under a hydrogen balloon for 4 h. The methanol had evaporated so the residue was dissolved/suspended in methanol (10 ml) and dichloromethane (10 ml) and the flask was then evacuated and filled with nitrogen three times to remove oxygen, finally the flask was evacuated and then filled with hydrogen gas from a balloon, then stirred at rt for 16 h. A further portion of 10% palladium on activated carbon (60 mg) was added and the flask was then evacuated and filled with nitrogen three times to remove oxygen, finally the flask was evacuated and then filled with hydrogen gas from a balloon, then stirred at rt for 25 h then diluted with methanol (50 ml) filtered through a thin layer of celite and washed with warm methanol:chloroform (1:1, 50 ml) the combined filtrates were evaporated. The residue was partitioned between sodium hydrogen carbonate (sat. aq. 150 ml) and ethyl acetate (150 ml) an insoluble yellow solid was filtered, then the layers separated. The aq layer was acidified by dropwise addition of hydrochloric acid (5M aq.) until a brown precipitate appeared (pH~5) which was filtered and dried in a vacuum oven. Purification by column chromatography on silica gel (20% methanol/chloroform) gave **17f** as a cream solid (60 mg, 0.136 mmol, 24%), mp 190-193 °C; ν_{max} (cm^{-1}) 3230 (NH), 3051 (NH), 1712 (C=O), 1629 (C=O); ^{1}H NMR (600 MHz, DMSO-d_6) δ ppm 4.64 (2H, br s, C(15)H_2), 5.10 (2H, br s, NH_2), 7.17 (1H, d, *J*=7.9 Hz, H(6)), 7.21 - 7.32 (2H, m, H(20) and H(5)), 7.40 (1H, s, H(3)), 7.43 - 7.58 (3H, m, H(12 and 13) and H(25)), 7.95 (2H, d, *J*=7.9 Hz, H(10 and 14)), 8.05 (1H, t, *J*=5.3 Hz, N(16)H), 8.37 - 8.47 (2H, m, H(24) and H(21)), 8.68 (1H, br s, H(26)), 9.24 (1H, br s, H(28)), 9.76 (1H, br s, N(7)H); ^{13}C NMR (150 MHz, DMSO-d_6) δ ppm 44.0 (C(15)), 106.0 (C(20)), 117.1 (C(3)), 117.5 (C(5)), 123.9 (C(25)), 125.7 (C(6)), 126.9 (C(11 and 13)), 127.2 (C(1)), 127.9 (C(10 and 14)), 129.1 (C(4)), 132.4 (C(23)), 132.8 (C(9)), 134.2 (C(24)), 142.3 (C(2)), 144.4 (C(12)), 148.1 (C(28)), 151.3 (C(26)), 159.4 (C(19)), 159.7 (C(21)), 162.4 (C(17)), 165.4 (C(8)), 168.0 (CO_2H); *m/z* (ES^-) 439 ($[M-H]^-$, 100%); HRMS $C_{24}H_{19}N_6O_3^-$ requires: 439.1519, found: 439.1531.

***N*-[2-amino-4-(hydroxymethyl)phenyl]-4-({[4-(pyridin-3-yl)pyrimidin-2-yl]amino}methyl)benzamide (17g)**



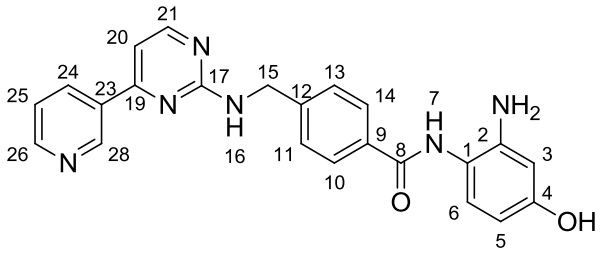
To a suspension of the silyl protected nitro compound **16d** (0.480 g, 0.841 mmol, 1 eq) in THF, methanol and water (10 ml of each) was added ammonium acetate (0.648 g, 8.41 mmol, 10 eq) and $SnCl_2$.water (1.138 g, 5.05 mmol, 6 eq). Then stirred at rt for 21 h, but TLC showed starting materials remained so the mixture was heated to 50 °C for 1 h. Tin salts were filtered and washed with ethyl acetate, and 10% methanol in dichloromethane. Then the filtrate was evaporated. Residue dissolved in ethyl acetate (200 ml) and washed with sat. aq. sodium hydrogen carbonate (100 ml) and brine (100 ml), dried $MgSO_4$ and evaporated to give a tan foamy solid which was purified by column chromatography on silica gel (10% to 20% methanol/dichloromethane) to give **17g** as a cream solid (0.165 g, 0.387 mmol, 46%), mp 127-129 °C; ν_{max} (cm^{-1}) 3265 (NH), 1647 (C=O); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 4.36 (2H, d, *J*=5.5 Hz, CH_2O), 4.64 (2H, d, *J*=5.9 Hz, CH_2N), 4.82 (2H, br s, NH_2), 5.02 (1H, t, *J*=5.5 Hz, OH), 6.52 (1H, d, *J*=7.8 Hz, H(5)), 6.73 (1H, s, H(3)), 7.07 (1H, d, *J*=7.8 Hz, H(6)), 7.25 (1H, d, *J*=4.9 Hz, H(20)), 7.42 - 7.56 (3H, m, H(11), H(13) and H(25)), 7.91 (2H, d, *J*=7.7 Hz, H(10) and H(14)), 7.98 (1H, t, *J*=5.9 Hz, CH_2NH), 8.40 (2H, d, *J*=4.9 Hz, H(24) and H(21)), 8.67 (1H, br s, H(26)), 9.23 (1H, br s, H(28)), 9.54 (1H, br s, CONH); ^{13}C NMR (150 MHz, DMSO-d_6) δ ppm 44.0 (CH_2N), 62.9 (CH_2O), 106.1 (C(20)), 114.2 (C(5)), 114.5 (C(3)), 121.9 (C(1)), 123.9 (C(25)), 126.4 (C(6)), 126.9 (C(11 and 13)), 127.8 (C(10 and 14)), 132.4 (C(23)), 133.0 (C(9)), 134.2 (C(24)), 140.8 (C(4)), 142.9 (C(2)), 144.1 (C(12)), 148.1 (C(28)), 151.3 (C(26)), 159.6 (C(21)), 161.0 (C(19)), 162.4 (C(17)), 165.2 (C=O); *m/z* (ES^+) 427 ($[M+H]^+$, 70%), 409 ($[M+H-water]^+$, 100%); HRMS $C_{24}H_{23}N_6O_2^+$ requires: 427.1882, found: 427.1872.

***N*-(2-amino-4-methoxyphenyl)-4-({[4-(pyridin-3-yl)pyrimidin-2-yl]amino}methyl)benzamide (18)**



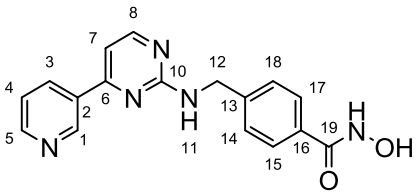
To a solution of benzoic acid **9** (0.306 g, 1.0 mmol, 1.0 eq), 4-methoxy-1,2-phenylene diamine (0.531 g, 1.1 mmol, 1.1 eq) and BOP (0.531 g, 1.2 mmol, 1.2 eq) in DMF (10 ml) was added triethylamine (0.28 ml, 2.0 mmol, 2.0 eq). The resulting solution was stirred at rt for 27 h under an nitrogen balloon, then diluted with ethyl acetate (50 ml), washed with brine (3 × 50 ml) and sodium hydrogen carbonate (sat. aq. 2 × 25 ml). The combined aq layers were extracted with ethyl acetate (25 ml) and the combined organic layers were washed with brine (2 × 25 ml), dried Na_2SO_4 and concentrated. Purification by column chromatography on silica gel (ethyl acetate) gave **18** as a cream solid (0.3 g, 0.70 mmol, 70%), mp 176-178 °C; ν_{max} (cm^{-1}) 3435 (NH), 3249 (NH), 1628 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 3.67 (3H, s, OCH_3), 4.65 (2H, d, *J*=5.9 Hz, C(15)H_2), 4.91 (2H, br s, NH_2), 6.18 (1H, dd, *J*=8.5, 2.5 Hz, H(5)), 6.35 (1H, d, *J*=2.5 Hz, H(3)), 6.99 (1H, d, *J*=8.5 Hz, H(6)), 7.27 (1H, d, *J*=5.0 Hz, H(20)), 7.41 - 7.60 (3H, m, H(11 and 13) and H(25)), 7.92 (2H, d, *J*=8.0 Hz, H(10 and 14), 8.02 (1H, t, *J*=5.9 Hz, N(16)H), 8.42 (2H, d, *J*=5.3 Hz, H(24) and H(21)), 8.69 (1H, d, *J*=3.3 Hz, H(26)), 9.25 (1H, br s, H(28)), 9.49 (1H, br s, N(7)H); ^{13}C NMR (150 MHz, DMSO-d_6) δ ppm 44.0 (C(15)), 54.9 (OCH_3), 100.7 (C(3)), 101.9 (C(5)), 106.1 (C(20)), 116.6 (C(1)), 123.9 (C(25)), 126.9 (C(11 and 13)), 127.7 (C(10 and 14)), 127.9 (C(6)), 132.4 (C(23)), 133.0 (C(9)), 134.2 (C(24)), 144.1 (C(12)), 144.6 (C(2)), 148.1 (C(28)), 151.3 (C(26)), 158.2 (C(4)), 159.2 (C(21)), 159.6 (C(19)), 162.4 (C(17)), 165.3 (C(8)); *m/z* (ES^-) 425 ([M-H]$^-$, 100%); HRMS $C_{24}H_{21}N_6O_2^-$ requires: 425.1726, found: 425.1732.

***N*-(2-amino-4-hydroxyphenyl)-4-({[4-(pyridin-3-yl)pyrimidin-2-yl]amino}methyl)benzamide (19)**



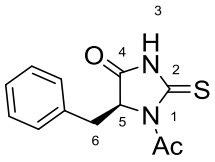
To a suspension of **18** (80 mg, 0.188 mmol, 1.0 eq) in dichloromethane (5 ml) at 0 °C was added boron tribromide (1M in dichloromethane, 0.56 ml, 0.56 mmol, 3.0 eq) dropwise over 5 min. The resulting mixture was stirred for 1 h at 0 °C then boron tribromide (1M in dichloromethane, 0.56 ml, 3.0 eq) was added and the reaction stirred at rt for 16 h. Another portion of boron tribromide (1M in dichloromethane, 0.56 ml, 3.0 eq) was added and the mixture stirred at rt for 7 h, then cooled to 0 °C and quenched with sodium hydrogen carbonate (sat. aq., 10 ml). A brown solid was filtered and washed with water (10 ml) and dichloromethane (10 ml) then dried. Purification by column chromatography on silica gel (7 to 10% methanol/dichloromethane) gave **19** as a beige solid (39 mg, 0.095 mmol, 50%), mp 223-225 °C; ν_{max} (cm^{-1}) 3325 (NH), 1640 (C=O); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 4.63 (2H, d, *J*=5.2 Hz, C(15)H_2), 4.74 (2H, br s, NH_2), 6.00 (4H, dd, *J*=8.4, 2.5 Hz, H(5)), 6.19 (1H, d, *J*=2.5 Hz, H(3)), 6.84 (1H, d, *J*=8.4 Hz, H(6)), 7.24 (1H, d, *J*=5.2 Hz, H(20)), 7.40 - 7.57 (3H, m, H(25) and H(11 and 13)), 7.89 (2H, d, *J*=8.2 Hz, H(10 and14)), 7.98 (1H, t, *J*=6.2 Hz, N(16)H), 8.40 (2H, br. d, *J*=5.2 Hz, H(21) and H(24)), 8.67 (1H, br. d, *J*=3.5 Hz, H(26)), 8.95 (1H, br s, OH), 9.23 (1H, br s, H(28)), 9.38 (1H, s, N(7)H); ^{13}C NMR (150 MHz, DMSO-d_6) δ ppm 44.0 (C(15)), 102.2 (C(3)), 103.7 (C(5)), 106.1 (C(20)), 115.2 (C(1)), 123.9 (C(25)), 126.8 (C(11 and 13)), 127.7 (C(10 and 14)), 127.9 (C(6)), 132.4 (C(23)), 133.1 (C(9)), 134.2 (C(24)), 144.0 (C(12)), 144.6 (C(2)), 148.1 (C(28)), 151.3 (C(26)), 156.2 (C(4)), 159.2 (C(21)), 159.6 (C(19)), 162.4 (C(17)), 165.3 (C(8)); *m/z* (ES^-) 411 ($[M-H]^-$, 100%); HRMS $C_{23}H_{19}N_6O_2^-$ requires: 411.1569, found: 411.1569.

***N*-hydroxy-4-({[4-(pyridin-3-yl)pyrimidin-2-yl]amino}methyl)benzamide (20)**



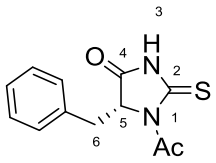
To a stirring solution of sodium hydroxide (0.25 g, 6.24 mmol, 8 eq) in 50% aqueous hydroxylamine (2.4 ml, 39.2 mmol, 50 eq) at 0 °C was added a solution of methyl ester **8** (0.25 g, 0.781 mmol, 1 eq) in THF (10 ml) dropwise over 10 min. Then solution was warmed to rt and stirred for a further 5 min then evaporated. Diluted with water (10 ml), cooled to 0 °C and slowly neutralised to pH 8 by dropwise addition of 2M hydrochloric acid. The precipitate was filtered, washed with water and dried. The resulting solid was purified by column chromatography on silica gel (8 to 20% methanol/dichloromethane) to give hydroxamic acid **20** as a cream solid (0.10 g, 0.332 mmol, 42%), mp 186-187 °C; ν_{max} (cm^{-1}) 3248 (NH), 1628 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 4.62 (2H, d, *J*=6.0 Hz, H(12)), 7.26 (1H, d, *J*=5.0 Hz, H(7)), 7.43 (2H, d, *J*=7.3 Hz, H(14 and 18)), 7.53 (1H, dd, *J*=7.0, 5.0 Hz, H(4)), 7.70 (2H, d, *J*=8.3 Hz, H(15 and 17)), 7.96 (1H, t, *J*=6.0 Hz, H(11)), 8.41 (2H, m, H(3) and H(8)), 8.68 (1H, d, *J*=3.8 Hz, H(5)), 8.97 (1H, br s, ONH), 9.23 (1H, br s, H(1)), 11.13 (1H, br s, OH); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 43.9 (C(12)), 106.1 (C(7)), 123.8 (C(4)), 126.9 (C(14 and 18)), 127.0 (C(15 and 17)), 131.1 (C(2)), 132.4 (C(16)), 134.2 (C(3)), 143.8 (C(13)), 148.0 (C(1)), 151.3 (C(5)), 159.5 (C(6) and C(8)), 162.4 (C(10)), 164.2 (C(19)); *m/z* (ES^+) 322 ($[M+H]^+$, 25%); HRMS $C_{24}H_{24}N_3OS_2^+$ requires: 322.1304, found: 322.1306.

(5*S*)-1-acetyl-5-benzyl-2-thiohydantoin ((*S*)-21a)[99]



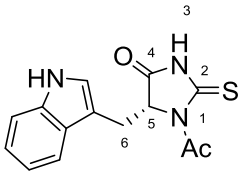
L-phenylalanine (1.65 g, 10 mmol) and ammonium thiocyanate (0.761 g, 10 mmol) were ground together with a mortar and pestle then transferred to a round bottomed flask and suspended in acetic anhydride (7.5 ml, 79.5 mmol). The mixture was stirred at 100 °C for 1 h then poured onto ice water (20 ml) and stored in a freezer overnight. The mixture was filtered and washed with cold water (30 ml) and the solid dried in a desiccator with phosphorus pentoxide as a drying agent to yield a cream solid. Recrystallization from ethanol and water gave **(*S*)-21a** as a white crystalline solid (1.17 g, 4.7 mmol, 47%), mp 167-169 °C (lit.[99] 170 °C); ν_{max} (cm^{-1}) 3083 (NH), 1732 (C=O), 1701 (C=O); ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 2.68 (3H, s, NCOCH_3), 3.10 (1H, dd, *J*=13.9, 2.7 Hz, C(6)*H*), 3.37 - 3.42 (1H, m, C(6)*H*`), 4.98 (1H, dd, *J*=5.6, 2.7 Hz, C(5)*H*), 6.85 - 7.08 (2H, m, Ar*H*), 7.2 – 7.3 (3H, m, Ar*H*), 12.42 (1H, br s, N*H*).

(5*R*)-1-acetyl-5-benzyl-2-thiohydantoin ((*R*)-21a)



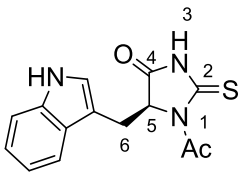
D-phenylalanine (1.65 g, 10 mmol) was reacted according to the above procedure for **(*S*)-21a** after recrystallization from ethanol and water gave **(*R*)-21a** as a white crystalline solid (0.777 g, 3.13 mmol, 31%), mp 163-165 °C; ν_{max} (cm^{-1}) 3083 (NH), 1744 (C=O), 1701 (C=O); ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 2.68 (3H, s, NCOCH_3), 3.10 (1H, dd, *J*=13.9, 2.7 Hz, C(6)*H*), 3.35 - 3.41 (1H, m, C(6)*H*`), 4.98 (1H, dd, *J*=5.8, 2.5 Hz, C(5)*H*), 6.89 - 7.05 (2H, m, Ar*H*), 7.13 - 7.39 (3H, m, Ar*H*), 12.42 (1H, br s, N*H*); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 27.3 (NCOCH_3), 34.4 (C(6)), 63.5 (C(5), 127.3 (1-aryl), 128.4 (2,6-aryl), 129.2 (3,5-aryl), 134.2 (4-aryl), 170.0, 172.6, 182.3 (2 C=O and C=S); *m/z* (EI^+) 248 (M^+, 69%); HRMS $C_{12}H_{12}N_2O_2S$ requires 248.0614, found 248.0607.

(5*R*)-1-acetyl-5-(1*H*-indol-3-ylmethyl)-2-thiohydantoin ((*R*)-21b)



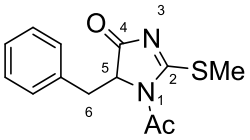
D-tryptophan (1.02 g, 4.97 mmol) and ammonium thiocyanate (0.378 g, 4.97 mmol) were ground together with a mortar and pestle then transferred to a round bottomed flask and suspended in acetic anhydride (3.3 ml, 35 mmol). The mixture was stirred at 100 °C for 10 min then poured onto ice water (20 ml) and stored in a freezer overnight. The mixture was triturated then filtered and washed with cold water (20 ml) and the solid dried in a desiccator with phosphorus pentoxide as a drying agent to yield an orange solid. Recrystallization from ethanol and water gave **(*R*)-21b** as a yellow solid (1.00 g, 3.51 mmol, 70%), mp 156-159 °C; ν_{max} (cm^{-1}) 3394 (NH), 1755 (C=O), 1675 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.68 (3H, s, NCOCH_3), 3.31 (1H, dd, *J*=14.8, 2.3 Hz, C(6)*H*), 3.55 (1H, dd, *J*=14.8, 5.5 Hz, C(6)*H*`), 4.99 (1H, dd, *J*=5.5, 2.3 Hz, C(5)*H*), 6.93 - 7.01 (2H, m, Ar*H*), 7.06 (1H, t, *J*=7.4 Hz, Ar*H*), 7.32 (1H, d, *J*=8.0 Hz, Ar*H*), 7.36 (1H, d, *J*=8.0 Hz, Ar*H*), 10.96 (1H, br s, indole N*H*), 12.30 (1H, s, N(3)*H*); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 24.7 (NCO*C*H$_3$), 27.5 (C(6)), 63.7 (C(5), 106.3, 111.4, 118.0, 118.6, 121.0, 124.1, 127.3, 135.8 (8 ArC), 170.1, 173.2, 182.5 (2 C=O and C=S); *m/z* (EI^+) 287 (M^+, 5%), 130 ([indole-CH_2]$^+$, 100%); HRMS $C_{14}H_{13}O_2N_3S$ requires: 287.0723, found: 287.0725.

(5*S*)-1-acetyl-5-(1*H*-indol-3-ylmethyl)-2-thiohydantoin ((*S*)-21b)[99]



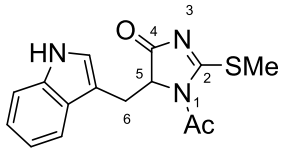
L-tryptophan (1.02 g, 4.97 mmol) and ammonium thiocyanate (0.378 g, 4.97 mmol) were ground together with a mortar and pestle then transferred to a round bottomed flask and suspended in acetic anhydride (3.3 ml, 35 mmol). The mixture was stirred at 100 °C for 25 min then poured onto ice water (20 ml) and stored in a freezer overnight. The mixture was filtered and washed with cold water (20 ml) and the solid dried in a desiccator with phosphorus pentoxide as a drying agent to yield an orange solid. Recrystallization from ethanol and water gave **(*S*)-21b** as a yellow solid (1.12 g, 3.91 mmol, 79%), mp 157-160 °C (lit.[99] 183-185 °C); ν_{max} (cm^{-1}) 3392 (NH), 1754 (C=O), 1673 (C=O); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 2.66 (3H, s, NCOCH_3), 3.29 (1H, dd, *J*=15.1, 2.2 Hz, C(6)*H*), 3.52 (1H, dd, *J*=14.8, 5.4 Hz, C(6)*H*`), 4.96 (1H, dd, *J*=5.7, 2.5 Hz, C(5)*H*), 6.91 - 6.97 (2H, m, Ar*H*), 7.03 (1H, t, *J*=7.3 Hz, Ar*H*), 7.30 (2H, d, *J*=7.9 Hz, Ar*H*), 7.34 (1H, d, *J*=7.9 Hz, Ar*H*), 10.94 (1H, br s, indole N*H*), 12.28 (1H, br s, N(3)*H*).

1-Acetyl-5-benzyl-2-(methylsulfanyl)-4,5-dihydro-1*H*-imidazol-4-one (22a)



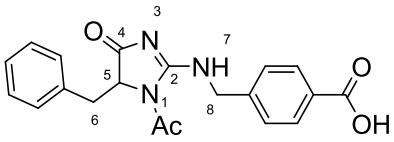
To a stirring mixture of thiohydantoin **(*R*)-21a** (0.70 g, 2.82 mmol) and anhydrous potassium carbonate (0.468 g, 3.38 mmol) in acetonitrile (35 ml) at rt was added iodomethane (0.263 ml, 4.23 mmol) dropwise. The resulting mixture was stirred at rt for 3.5 h under nitrogen, then filtered and then filtrate evaporated. The resulting cream solid was purified by column chromatography (ethyl acetate:hexane, 1:9 to 2:1) to give **22a** as a white solid (0.203 g, 0.774 mmol, 27%), mp 142-143 °C; ν_{max} (cm^{-1}) 1725 (C=O), 1698 (C=O); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 2.24 (3H, s, SCH_3), 2.39 (3H, s, NCOCH_3), 3.17 (1H, dd, *J*=14.3, 2.7 Hz, C(6)*H*), 3.35 (1H, dd, *J*=14.3, 6.0 Hz, C(6)*H*`), 4.96 (1H, dd, *J*=6.0, 2.8 Hz, C(5)*H*), 6.97 - 7.06 (2H, m, Ar*H*), 7.22 (3H, s, Ar*H*); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 15.7 (SCH_3), 23.6 (NCOCH_3), 36.1 (C(6)), 64.1 (C(5), 127.1 (1-aryl), 128.2 (2,6-aryl), 129.4 (3,5-aryl), 134.0 (4-aryl), 168.5, 184.5, 185.4 (2 C=O and C=N); *m/z* (EI^+) 262 (M^+, 12%); HRMS $C_{13}H_{14}O_2N_2S$ requires: 262.0771, found: 262.0765.

1-Acetyl-5-(1*H*-indol-3-ylmethyl)-2-(methylsulfanyl)-4,5-dihydro-1*H*-imidazol-4-one (22b)



Thiohydantoin **(*R*)-21b** (0.907 g, 3.38 mmol) was reacted according to the above procedure for **22a**, purification by column chromatography (ethyl acetate:hexane, 1:1 to 2:1) gave **22b** as a cream solid (0.610 g, 2.03 mmol, 60%), mp 187-190 °C; ν_{max} (cm^{-1}) 3234 (NH), 1725 (C=O), 1698 (C=O); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 2.22 (3H, s, SCH_3), 2.39 (3H, s, NCOCH_3), 3.35 (1H, dd, *J*=15.4, 2.9 Hz, C(6)*H*), 3.47 (1H, dd, *J*=15.4, 5.8 Hz, C(6)*H*`), 4.94 (1H, dd, *J*=5.8, 3.0 Hz, C(5)*H*), 6.93 (1H, t, *J*=7.5 Hz, Ar*H*), 6.96 (1H, d, *J*=2.4 Hz, Ar*H*), 7.03 (1H, t, *J*=7.5 Hz, Ar*H*), 7.29 (1H, d, *J*=8.0 Hz, Ar*H*), 7.40 (1H, d, *J*=8.0 Hz, Ar*H*), 10.90 (1H, br s, N*H*); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 15.6 (SCH_3), 23.6 (NCO*C*H_3), 26.4 (C(6)), 64.1 (C(5), 106.4, 111.3, 118.3, 118.5, 121.0, 124.1, 127.2, 135.7 (8 ArC), 168.5, 185.1, 185.3 (2 C=O and C=N); *m/z* (EI^+) 301 (M^+, 10%), 130 ([indole-CH_2]$^+$, 100%); HRMS $C_{15}H_{15}N_3O_2S$ requires: 301.0880, found: 301.0878.

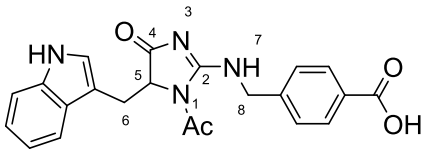
4-({[1-acetyl-5-benzyl-4-oxo-4,5-dihydro-1*H*-imidazol-2-yl]amino}methyl)benzoic acid (23a)



To a solution of **22a** (179 mg, 0.682 mmol) in ethanol (8 ml) was added 4-(methylamino)benzoic acid (103 mg, 0.682 mmol). The resulting mixture was stirred at reflux under nitrogen for 18 h. The solution was cooled to rt, then cooled in a refrigerator before being filtered and the solid dried under vacuum to give **23a** as a cream solid (122 mg, 0.335 mmol, 49%), mp 232-234 °C; ν_{max} (cm^{-1}) 3263 (NH/CO_2H), 1688 (C=O); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 2.42 (9H, s, NCOCH_3), 3.14 (1H, dd, *J*=14.2, 1.9 Hz, C(6)*H*), 3.30 (1H, dd, *J*=14.2, 5.7 Hz, C(6)*H*`), 4.37 (1H, dd, *J*=15.8, 6.0 Hz, C(8)*H*), 4.52 (1H, dd, *J*=15.8, 6.6 Hz, C(8)*H*`), 4.77 (1H, dd, *J*=5.7, 1.9 Hz, C(5)*H*), 6.91 (2H, d, *J*=8.2 Hz, Ar*H*), 6.99 - 7.08 (2H, m, Ar*H*), 7.18 - 7.32 (3H, m, Ar*H*), 7.77 (2H, d, *J*=8.2 Hz, Ar*H*), 9.28 (1H, t, *J*=6.3 Hz, N*H*); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 24.2 (NCO*C*H_3), 35.7 (C(6)), 45.4 (C(8)), 63.1 (C(5), 126.6, 126.8, 128.1, 129.2, 129.6, 134.4, 142.9 (7 ArC), 167.2, 167.6, 171.2, 183.4 (3 C=O and C=N);

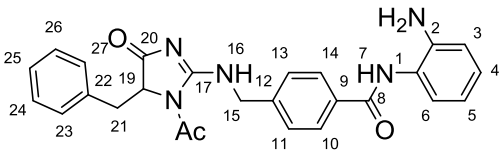
m/z (ES^+) 366 ($[M+H]^+$, 100%); HRMS $C_{20}H_{20}N_3O_4$ requires: 366.1454, found: 366.1448.

4-({[1-acetyl-5-(1*H*-indol-3-ylmethyl)-4-oxo-4,5-dihydro-1*H*-imidazol-2-yl]amino}methyl)benzoic acid (23b)



22b (0.60 g, 1.99 mmol) was reacted according to the above procedure for **23a**, filtration gave **23b** as a tan solid (0.295 g, 0.729 mmol, 37%) and the mother liquor was evaporated and the residue re-crystallised from ethanol to give further **23b** as a tan solid (0.129 g, 0.320 mmol, 16%) overall yield 53%, mp163-166 °C; ν_{max} (cm^{-1}) 3315 (NH), 1728 (C=O), 1674 (C=O), 1663 (C=O);^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 2.46 (3H, s, NCOC*H*$_3$), 3.31 (1H, dd, *J*=15.1, 2.2 Hz, C(6)*H*), 3.45 (6H, dd, *J*=15.1, 5.4 Hz, C(6)*H*`), 4.32 (1H, dd, *J*=15.8, 5.7 Hz, C(8)*H*), 4.49 (1H, dd, *J*=15.8, 6.9 Hz, C(8)*H*`), 4.75 (1H, dd, *J*=5.4, 2.2 Hz, C(5)*H*), 6.74 (2H, d, *J*=8.0 Hz, Ar*H*), 6.92 (1H, t, *J*=7.3 Hz, Ar*H*), 6.99 (1H, d, *J*=2.0 Hz, Ar*H*), 7.04 (1H, t, *J*=7.4 Hz, Ar*H*), 7.36 (1H, d, *J*=8.2 Hz, Ar*H*), 7.41 (1H, d, *J*=7.9 Hz, Ar*H*), 7.74 (2H, d, *J*=8.2 Hz, Ar*H*), 9.20 (1H, t, *J*=6.3 Hz, N(7)*H*), 11.00 (1H, br s, indole N*H*); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 24.2 (NCO*C*H$_3$), 26.2 (C(6)), 45.3 (C(8)), 63.3 (C(5), 106.6, 111.3, 118.3, 118.5, 120.8, 124.3, 126.2, 127.5, 129.3, 129.3, 135.9, 142.9 (12 ArC), 167.2, 167.8, 171.2, 184.1 (3 C=O and C=N); *m/z* (ES^-) 403 ($[M-H]^-$, 95%); HRMS $C_{22}H_{19}N_4O_4$ requires: 403.1406, found: 403.1412.

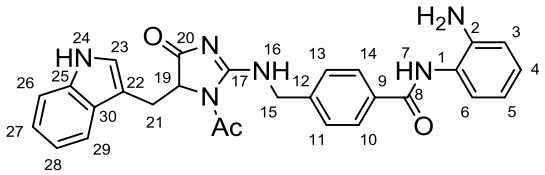
4-({[1-acetyl-5-benzyl-4-oxo-4,5-dihydro-1*H*-imidazol-2-yl]amino}methyl)-*N*-(2-aminophenyl)benzamide (24a)



To a solution of the acid **23a** (114 mg, 0.312 mmol) and BOP (165 mg, 0.374 mmol) in anhydrous DMF (1.4 ml) was added triethylamine (174 µl, 1.25 mmol) and then stirred at rt for 15 min under nitrogen. To this solution was added phenylene-1,2-diamine (40 mg, 0.374 mmol) and then the solution stirred at rt under nitrogen for 3.5 h. The reaction mixture was then diluted with ethyl acetate (50 ml) and washed with water (25 ml), sodium hydrogen carbonate (25 ml), and brine (25 ml). Dried $MgSO_4$ and evaporated to give an orange solid. (If an oil was isolated at this point the oil was

dissolved in ethyl acetate and washed with sat. aq. lithium chloride, or triturated with diethyl ether to remove DMF.) Purification by column chromatography (4% methanol/dichloromethane) gave benzamide **24a** as a white solid (21 mg, 0.0464 mmol, 15%), mp 235-236 °C; ν_{max} (cm^{-1}) 3283 (NH), 1720 (C=O), 1671 (C=O); ^{1}H NMR (600 MHz, DMSO-d_6) δ ppm 2.44 (3H, s, COCH_3), 3.16 (1H, dd, *J*=14.0, 2.3 Hz, H(21)), 3.31 (1H, dd, *J*=14.0, 5.6 Hz, H(21)'), 4.40 (1H, dd, *J*=15.6, 5.6 Hz, H(15)), 4.54 (1H, dd, *J*=15.6, 6.6 Hz, H(15)'), 4.80 (1H, dd, *J*=5.6, 2.3 Hz, H(19)), 4.91 (2H, br s, NH_2), 6.61 (1H, t, *J*=7.3 Hz, H(5)), 6.79 (1H, d, *J*=7.2 Hz, H(3)), 6.94 - 7.00 (3H, m, H(11 and 13) and H(4)), 7.07 (2H, d, *J*=7.2 Hz, H(23 and 27)), 7.17 (1H, d, *J*=7.5 Hz, H(6)), 7.28 (2H, t, *J*=7.5 Hz, H(24 and 26)), 7.32 (1H, t, *J*=7.2 Hz, H(24)), 7.84 (2H, d, *J*=7.5 Hz, H(10 and 14)), 9.30 (1H, t, *J*=6.4 Hz, H(16)), 9.64 (1H, s, H(7)); ^{13}C NMR (150 MHz, DMSO-d_6) δ ppm 24.3 (CH_3), 35.8 (C(21)), 45.4 (C(15)), 63.1 (C(19)), 116.1 (C(3)), 116.3 (C(5)), 123.3 (C(1)), 126.5 (C(11 and 13)), 126.6 (C(4)), 126.8 (C(6)), 127.0 (C(25)), 127.7 (C(10 and 14)), 128.3 (C(23 and 27)), 129.6 (C(24 and 26)), 133.2 (C(9)), 134.4 (C(22)), 141.4 (C(12)), 143.2 (C(2)), 165.1 (C(17)), 167.6 (C(8)), 171.3 (NAc), 183.5 (C(20)); *m/z* (ES^+) 456 ($[M+H]^+$, 80%); HRMS $C_{26}H_{26}N_5O_3$, requires: 456.2036, found: 456.2017.

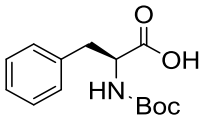
4-({[1-acetyl-5-(1*H*-indol-3-ylmethyl)-4-oxo-4,5-dihydro-1*H*-imidazol-2-yl]amino}methyl)-*N*-(2-aminophenyl)benzamide (24b)



Acid **23b** (240 mg, 0.593 mmol) was reacted according to the above procedure for **24a**, purification by column chromatography (6% methanol/dichloromethane) gave a cream solid (260 mg), recrystallization from methanol and dichloromethane then gave benzamide **24b** as a cream crystalline solid (182 mg, 0.367 mmol, 62%), mp 168-170 °C; ν_{max} (cm^{-1}) 3267 (NH), 1720 (C=O), 1676 (C=O); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 2.45 (3H, s, COCH_3), 3.31 (1H, dd, *J*=15.1, 2.4 Hz, H(21)), 3.46 (1H, dd, *J*=15.1, 5.4 Hz, H(21)'), 4.36 (1H, dd, *J*=15.6, 6.0 Hz, H(15)), 4.49 (1H, dd, *J*=15.6, 6.8 Hz, H(15)'), 4.76 (1H, dd, *J*=5.4, 2.4 Hz, H(19)), 4.90 (2H, br s, NH_2), 6.61 (1H, t, *J*=8.0 Hz, H(5)), 6.79 (1H, dd, *J*=8.0, 1.1 Hz, H(3)), 6.84 (2H, d, *J*=8.0 Hz, H(11 and 13)), 6.92 - 7.00 (3H, m, H(27), H(28) and H(23)), 7.05 (1H, t, *J*=8.0 Hz, H(4)), 7.19 (1H, d, *J*=7.7 Hz, H(6)), 7.38 (1H, d, *J*=8.0 Hz, H(26)), 7.43 (1H, d, *J*=8.0 Hz, H(29)), 7.79 (2H, d, *J*=8.0 Hz, H(10 and 14)), 9.22 (1H, t, *J*=6.4 Hz, H(16)), 9.55 (1H, s, H(7)),

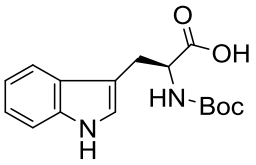
10.96 (1H, br. d, *J*=1.6 Hz, H(24)); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 24.2 (CH_3), 26.2 (C(21)), 45.3 (C(15)), 63.3 (C(19)), 106.6 (C(22)), 111.4 (C(26)), 116.1 (C(3)), 116.3 (C(5)), 118.3 (C(29)), 118.5 (C(28)), 120.9 (C(27)), 123.4 (C(1)), 124.2 (C(23)), 126.2 (C(11 and 13), 126.5 (C(4)), 126.6 (C(6)), 127.5 (C(30)), 127.7 (C(10 and 14)), 133.2 (C(9)), 135.8 (C(25)), 141.3 (C(12)), 143.1 (C(2)), 165.2 (C(17)), 167.7 (C(8)), 171.2 (NAc), 184.1 (C(20)); *m/z* (ES$^+$) 495 ([M+H]$^+$, 100%); HRMS $C_{28}H_{27}N_6O_3$, requires: 495.2145, found: 495.2129.

_N_-(*tert*-butoxycarbonyl)-L-phenylalanine (25a)[102]



To a solution of L-phenylalanine (1.6 g, 9.69 mmol) in THF:water (1:1, 40 ml), was added di-*tert*-butyl dicarbonate (2.33 g, 10.7 mmol), then sodium hydroxide (0.853 g, 21.3 mmol) was added and the reaction was stirred at rt for 16 h under nitrogen. The THF was evaporated and dichloromethane (60 ml) was added then 10% aq. hydrochloric acid was added dropwise whilst stirring vigorously until the precipitate ceased forming (~pH 4). The organic layer was separated, dried $MgSO_4$, and evaporated to give a colourless oil which was then dissolved in sat. aq. sodium hydrogen carbonate (50 ml) and washed with dichloromethane (50 ml). The aqueous layer was then acidified by dropwise addition of 2M hydrochloric acid to ~pH 4 and extracted with dichloromethane (50 ml), dried, $MgSO_4$ to yield **25a** as a clear oil (2.53 g, 9.54 mmol, 98%); ν_{max} (cm^{-1}) 3200-2800 br. (NH and OH), 1714 (C=O); ^{1}H NMR (1:2 mixture of rotamers) (500 MHz, chloroform-*d*) δ ppm 1.29 (3H, s, C(C*H*$_3$)$_3$), 1.44 (6H, s, C(C*H*$_3$)$_3$), 2.93 (m) and 3.08 (dd, *J*=13.7, 6.1 Hz) (1H, PhC*H*H`), 3.20 (1H, m, PhCH*H*`), 4.41 (m) and 4.62 (m) (1H, C*H*(N)CO), 4.95 (d, *J*=7.4 Hz) and 6.41 (m) (1H, N*H*), 7.12 - 7.37 (5H, m, Ar*H*), 9.57 (1H, br s, CO_2H); *m/z* (CI$^+$) 266 ([M+H]$^+$, 32%).

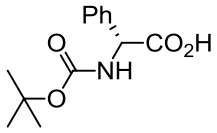
_N_-(*tert*-butoxycarbonyl)-L-tryptophan (25b)[103]



L-tryptophan (1.6 g, 7.84 mmol) was suspended in methanol (60 ml) and triethylamine (2.23 ml, 16.0 mmol) was added followed by di-*tert*-butyl dicarbonate (2.27 g, 10.4 mmol) then stirred at rt for 16 h under nitrogen by which time all solids

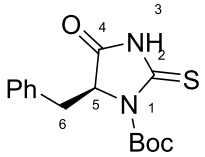
had dissolved. Solvents were evaporated, and then the residue was dissolved in sat. aq. sodium hydrogen carbonate (200 ml) and washed with dichloromethane (100 ml). The aqueous layer was acidified by dropwise addition of 5M hydrochloric acid to pH 4 and extracted with dichloromethane (200 ml). This dichloromethane was dried $MgSO_4$ and evaporated to give **25b** as a white solid (2.13 g, 7.0 mmol, 89%), mp 130-131 °C (lit.[103] 138-139 °C); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 1.32 (9H, s, C(CH_3)$_3$), 2.96 (1H, dd, *J*=14.5, 9.5 Hz, PhC*H*H`), 3.12 (1H, dd, *J*=14.5, 4.6 Hz, PhCH*H*`), 4.13 (1H, app. td, *J*=8.5, 4.6 Hz, C*H*(N)CO), 6.94 (1H, d, *J*=8.2 Hz, BocN*H*), 6.97 (1H, t, *J*=7.5 Hz, Ar*H*), 7.05 (1H, t, *J*=7.5 Hz, Ar*H*), 7.13 (1H, br s, Ar*H*), 7.32 (1H, d, *J*=8.0 Hz, Ar*H*), 7.51 (1H, d, *J*=8.0 Hz, Ar*H*), 10.81 (1H, br s, indole N*H*), 12.51 (1H, br s, CO_2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 26.8 C(*C*H$_3$)$_3$), 28.2 (CH_2), 54.5 (CHN), 78.0 (O*C*(CH$_3$)$_3$), 110.2, 111.4, 118.1, 118.3, 120.9, 123.6, 127.2, 136.1 (8 AcC), 155.4 (CO_2N), 173.9 (CO_2H).

_N_-(_tert_butoxycarbonyl) D-phenylglycine (25c)[144]



To a mixture of D-phenylglycine (1.6 g, 10.6 mmol) and sodium hydrogen carbonate (1.33 g, 15.9 mmol) was added THF:water (1:1, 60 ml) then di-*tert*-butyl dicarbonate and stirred at rt for 17 h. The THF was evaporated, then diluted with 200 ml water, washed with 100 ml diethyl ether. The aqueous layer was acidified with 3M hydrochloric acid to pH 3 then extracted with diethyl ether (3 × 80 ml). These three were combined washed with brine, dried over $MgSO_4$ and evaporated to give **25c** as a white solid (2.54 g, 10.0 mmol, 95%), mp 87-88 °C (lit. 87-89.5 °C)[145]; ^{1}H NMR (400 MHz, chloroform-*d*) 2:1 ratio of rotamers δ ppm 1.24 (6H, s, (C(C*H*$_3$)$_3$)) 1.46 (3H, s, (C(C*H*$_3$)$_3$)), 5.16 (0.66H, d, *J*=4.5 Hz, NCH) 5.32 - 5.42 (0.33H, m, NCH), 5.53 - 5.64 (0.33H, m, NH) 8.11 (0.66H, d, *J*=4.5 Hz, NH), 7.29 - 7.52 (5H, m, Ph), 9.94 (1H, br s, CO_2H); ^{13}C NMR (125 MHz, chloroform-*d*) δ ppm 28.0 (C(*C*H$_3$)$_3$), 58.9 (CH), 81.7 (O*C*(CH$_3$)$_3$), 127.3, 128.0, 128.5, 138.4 (4 ArC), 157.1 (NCOO), 173.5 (CO_2H).

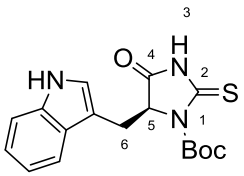
(5*S*)-1-(*tert*-butoxycarbonyl)-5-benzyl-2-thiohydantoin (26a)[101]



To a solution of *N*-(*tert*-butoxycarbonyl)-L-phenylalanine **25a** (100 mg, 0.377

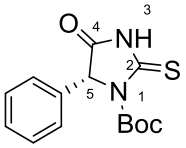
mmol, 1.0 eq) in acetonitrile (10 ml) was added ethoxycarbonyl isothiocyanate (53 µl, 0.452 mmol, 1.2 eq) and pyridine (37 µl, 0.452 mmol, 1.2 eq). The resulting solution was stirred at rt for 18 h, then the solvent was evaporated and the residue was purified by column chromatography (ethyl acetate:hexane, 1:1) to give **26a** as a white solid (104 mg, 0.338 mmol, 90%), mp 113-115 °C; ν_{max} (cm^{-1}) 3239 (NH), 1745 (C=O); ^{1}H NMR (500 MHz, chloroform-*d*) δ ppm 1.63 (9H, s, C(C*H*$_3$)$_3$), 3.29 (1H, dd, *J*=14.2, 2.5 Hz, C(6)*H*), 3.49 (1H, dd, *J*=13.9, 5.7 Hz, C(6)*H*`), 4.81 (1H, dd, *J*=5.4, 2.8 Hz, C(5)*H*), 7.08 (2H, dd, *J*=6.5, 2.7 Hz, Ar*H*), 7.19 - 7.33 (3H, m, Ar*H*), 8.54 (1H, br s, N*H*); ^{13}C NMR (125 MHz, chloroform-*d*) δ ppm 28.2 (C(*C*H$_3$)$_3$), 36.0(C(6)), 64.5(C(5), 85.6 (O*C*(CH$_3$)$_3$), 127.9 (1-aryl), 128.9 (2,6-aryl), 129.6 (3,5-aryl), 133.0 (4-aryl), 148.6 (CO_2N), 171.0, 178.2 (C=O and C=S); *m/z* (EI^+) 306 (M^+, 7%), 206 ([M-Boc]$^+$, 95%); HRMS $C_{15}H_{18}N_2O_3S$ requires: 306.1033, found: 306.1038.

(5*S*)-1-(*tert*-butoxycarbonyl)-5-(1*H*-indol-3-ylmethyl)-2-thiohydantoin (26b)



N-(*tert*-butoxycarbonyl)-L-tryptophan **25b** (2.11 g, 6.93 mmol) was reacted according to the above procedure for **26a**. Purification by column chromatography (ethyl acetate:hexane, 1:2) gave **26b** as a white solid (1.98 g, 5.72 mmol, 82%), mp 85-87 °C; ν_{max} (cm^{-1}) 3354 (NH), 1736 (C=O); ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 1.64 (9H, s, C(C*H*$_3$)$_3$), 3.53 (1H, dd, *J*=15.0, 2.8 Hz, C(6)*H*), 3.63 (1H, dd, *J*=15.1, 5.3 Hz, C(6)*H*`), 4.81 (1H, dd, *J*=5.3, 2.9 Hz, C(5)*H*), 6.93 (1H, d, *J*=2.0 Hz, Ar*H*), 7.10 (1H, t, *J*=7.2 Hz, Ar*H*), 7.17 (1H, t, *J*=7.2 Hz, Ar*H*), 7.31 (1H, d, *J*=8.0 Hz, Ar*H*), 7.56 (1H, d, *J*=8.0 Hz, Ar*H*), 8.12 (1H, br s, indole N*H*), 8.35 (1H, br s, N(3)*H*); ^{13}C NMR (125 MHz, chloroform-*d*) δ ppm 26.1 (C(*C*H$_3$)$_3$), 28.2 (C(6)), 64.4 (C(5), 85.5 (O*C*(CH$_3$)$_3$), 107.1, 111.3, 118.7, 120.0, 122.4, 123.7, 127.4, 135.9 (8 ArC), 148.8 (CO_2N), 171.5, 178.6 (C=O and C=S); *m/z* (EI^+) 345 (M^+, 12%), 130 ([indole-CH_2]$^+$, 97%); HRMS $C_{17}H_{19}O_3N_3S$ requires: 345.1142, found: 345.1144.

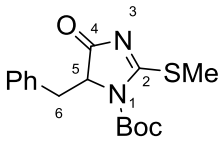
(5*R*)-1-(*tert*-butoxycarbonyl)-5-phenyl-2-thiohydantoin (26c)



N-Boc D-Phenylglycine **25c** (2.5 g, 9.95 mmol, 1.0 eq) was reacted according to

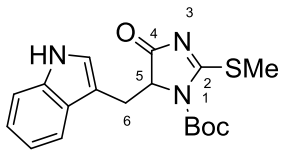
the procedure for **26a**. Purification by column chromatography (hexane:ethyl acetate, 3:1 to ethyl acetate) gave a sticky white solid (some pyridine) which was recrystallized from chloroform to give **26c** as a white solid (1.71 g, 5.84 mmol, 59%), mp 172-173 °C; ν_{max} (cm^{-1}) 3111 (NH), 1740 (C=O); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 1.17 (9H, s, C(CH_3)$_3$), 5.62 (1H, s, C*H*), 7.23 (2H, d, *J*=6.9 Hz, Ar*H*), 7.34 - 7.47 (3H, m, Ar*H*), 12.63 (1H, br s, N*H*); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 27.2 (C(*C*H_3)$_3$), 66.6 (CH), 83.3 (*C*(CH$_3$)$_3$), 126.4 (1-aryl), 128.7 (2,6-aryl), 129.0 (3,5-aryl), 134.9 (4-aryl), 147.5 (NC=OO), 171.3 (C=S), 180.9 (HNC=O); *m/z* (EI$^+$) 292 (M$^+$, 3%), 192 ([M-Boc]$^+$, 100%); HRMS $C_{14}H_{16}N_2O_3S^+$ requires: 292.0876, found: 292.0870.

1-(*tert*-butoxycarbonyl)-5-benzyl-2-(methylsulfanyl)-4,5-dihydro-1*H*-imidazol-4-one (27a)



Thiohydantoin **26a** (0.90 g, 2.90 mmol) was reacted according to the procedure for **22a**, purification by column chromatography (ethyl acetate:hexane, 1:2) gave **27a** as a white solid (0.586 g, 1.83 mmol, 63%), mp 105-107 °C; ν_{max} (cm^{-1}) 1722 (C=O); ^{1}H NMR (500 MHz, chloroform-*d*) δ ppm 1.61 (9H, s, C(CH_3)$_3$), 2.37 (3H, s, SCH_3), 3.32 (1H, dd, *J*=13.9, 2.5 Hz, C(6)*H*), 3.42 (1H, dd, *J*=13.9, 6.0 Hz, C(6)*H*), 4.52 (1H, dd, *J*=6.0, 2.5 Hz, C(5)*H*), 7.02 - 7.10 (2H, m, Ar*H*), 7.18 - 7.25 (3H, m, Ar*H*); ^{13}C NMR (125 MHz, chloroform-*d*) δ ppm 16.2 (SCH$_3$), 28.2 (C(*C*H$_3$)$_3$), 36.1 (C(6)), 64.5 (C(5), 85.7 (O*C*(CH$_3$)$_3$), 127.4 (1-aryl), 128.6 (2,6-aryl), 129.5 (3,5-aryl), 133.8 (4-aryl), 148.4 (CO_2N), 184.7, 186.0 (C=O and C=N); *m/z* (ES$^+$) 321 ([M+H]$^+$, 100%); HRMS $C_{16}H_{21}N_2O_3S$ requires: 321.1264, found: 321.1273.

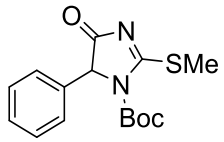
1-(*tert*-butoxycarbonyl)-5-(1*H*-indol-3-ylmethyl)-2-(methylsulfanyl)-4,5-dihydro-1*H*-imidazol-4-one (27b)



Thiohydantoin **26b** (1.85 g, 5.36 mmol) was reacted according to the procedure for **22a**, purification by column chromatography (ethyl acetate:hexane, 2:3 to 1:1) gave **27b** as a cream solid (1.35 g, 3.76 mmol, 70%), mp 96-98 °C; ν_{max} (cm^{-1}) 3312 (NH), 1720 (C=O); ^{1}H NMR (500 MHz, chloroform-*d*) δ ppm 1.60 (9H, s, C(CH_3)$_3$), 2.33 (3H, s, SCH_3), 3.49 (1H, dd, *J*=15.1, 3.0 Hz, C(6)*H*), 3.64 (1H, dd, *J*=15.0, 5.5 Hz,

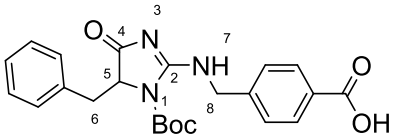
C(6)*H*`), 4.60 (1H, dd, *J*=5.5, 3.0 Hz, C(5)*H*), 6.95 (1H, d, *J*=2.4 Hz, Ar*H*), 7.07 (1H, t, *J*=7.5 Hz, Ar*H*), 7.14 (1H, t, *J*=7.6 Hz, Ar*H*), 7.30 (1H, d, *J*=8.0 Hz, Ar*H*), 7.53 (1H, d, *J*=8.0 Hz, Ar*H*), 8.26 (1H, br s, N*H*); ^{13}C NMR (125 MHz, chloroform-*d*) δ ppm 16.2 (SCH_3), 26.0 ($C(CH_3)_3$), 28.2 (C(6)), 64.8 (C(5)), 85.8 ($OC(CH_3)_3$), 107.9, 111.2, 118.5, 119.6, 122.0, 123.5, 127.8, 135.9 (8 ArC), 148.5 (CO_2N), 185.4 and 186.1 (C=O and C=N); *m/z* (ES^+) 382 ($[M+Na]^+$, 90%); HRMS $C_{18}H_{21}N_3O_3NaS$ requires: 382.1201, found: 382.1188.

1-(*tert*-butoxycarbonyl)-5-phenyl-2-(methylsulfanyl)-4,5-dihydro-1*H*-imidazol-4-one (27c)



To a stirring mixture of thiohydantoin **26c** (0.25 g, 0.855 mmol, 1.0 eq) and anhydrous potassium carbonate (0.141 g, 1.03 mmol, 1.2 eq) in acetonitrile (10 ml) at rt was added iodomethane (80 µL, 1.28 mmol, 1.5 eq) dropwise. The resulting mixture was stirred vigorously at rt for 2.5 h under nitrogen, then evaporated. The resulting residue was dissolved/suspended in ethyl acetate (20 ml) and filtered then the filtrate was evaporated to give **27c** as a white solid (0.259 g, 0.845 mmol, 99%), mp 145-147 °C; ν_{max} (cm^{-1}) 1737 (C=O), 1713 (C=O); ^{1}H NMR (500 MHz, chloroform-*d*) δ ppm 1.26 (9H, s, $C(CH_3)_3$), 2.67 (3H, s, SCH_3), 5.18 (1H, s, CH), 7.15 - 7.23 (2H, m, ArH), 7.30 - 7.39 (3H, m, ArH); ^{13}C NMR (125 MHz, chloroform-*d*) δ ppm 16.4 (CH_3), 27.7 ($C(CH_3)_3$), 67.6 (CH_2), 85.4 ($OC(CH_3)_3$), 126.4 (1-aryl), 128.7 (2,6-aryl), 129.0 (3,5-aryl), 134.5 (4-aryl), 148.4 (NC=OO), 183.0 (C=N), 186.6 (N*C*=OC); *m/z* (EI^+) 306 (M^+, 3%), 205 ($[M-Boc]^+$, 100%); HRMS $C_{15}H_{18}O_3N_2S^+$ requires: 306.1033, found: 306.1036.

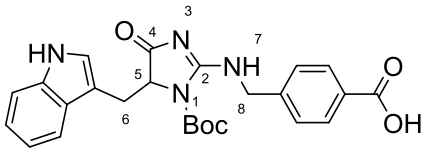
4-({[1-(*tert*-butoxycarbonyl)-5-benzyl-4-oxo-4,5-dihydro-1*H*-imidazol-2-yl]amino}methyl)benzoic acid (28a)



To a solution of **27a** (0.550 g, 1.72 mmol) in ethanol (15 ml) was added 4-(methylamino)benzoic acid (0.260 g, 1.72 mmol). The resulting mixture was stirred at reflux under nitrogen for 18 h. The solvent was evaporated and the residue dissolved in

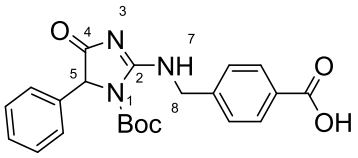
ethyl acetate (50 ml) then washed with water (50 ml) and brine (50 ml). The organic layer was dried $MgSO_4$ and evaporated. Purification by column chromatography (ethyl acetate) gave **28a** as a cream solid (0.238 g, 0.562 mmol, 33%), mp 137-140 °C; ν_{max} (cm^{-1}) 3386 (NH/CO_2H), 1702 (C=O); ^{1}H NMR (500 MHz, chloroform-*d*) δ ppm 1.62 (9H, br s, C(CH_3)$_3$), 3.22 - 3.40 (2H, m, , C(6)H_2), 4.28 - 4.44 (1H, m, C(8)*H*), 4.49 (1H, br s, C(8)*H*`), 4.77 (1H, dd, *J*=14.8, 6.6 Hz, C(5)*H*), 6.96 (2H, d, *J*=7.3 Hz, Ar*H*), 7.10 (2H, d, *J*=6.3 Hz, Ar*H*), 7.15 - 7.34 (3H, m, Ar*H*), 7.94 (2H, d, *J*=7.3 Hz, Ar*H*), 8.43 (1H, br s, N*H*), 8.95 (1H, br s, CO_2H); ^{13}C NMR (125 MHz, chloroform-*d*) δ ppm 28.3 (C(CH_3)$_3$), 35.8 (C(6)), 46.4 (C(8)), 63.5 (C(5)), 85.7 (O*C*(CH_3)$_3$), 127.2, 127.4, 128.6, 128.9, 129.7, 130.6, 134.1, 142.2 (8 ArC), 167.8, 170.5, 174.1, 184.3 (3 C=O and C=N); *m/z* (ES^-) 422 ([M-H]$^-$, 100%); HRMS $C_{23}H_{24}N_3O_5$ requires: 422.1716, found: 422.1707.

4-({[1-(*tert*-butoxycarbonyl)-5-(1*H*-indol-3-ylmethyl)-4-oxo-4,5-dihydro-1*H*-imidazol-2-yl]amino}methyl)benzoic acid (28b)



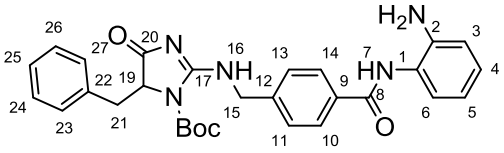
To a solution of **27b** (1.30 g, 3.62 mmol) in ethanol (30 ml) was added 4-(methylamino)benzoic acid (0.547 g, 3.62 mmol). The resulting mixture was stirred at reflux under nitrogen for 4 h. The solvent was evaporated and the resulting yellow solid purified by column chromatography (6% methanol/dichloromethane) to give **28b** as a cream solid (0.997 g, 2.16 mmol, 60%), mp 187-190 °C; ν_{max} (cm^{-1}) 3271 (NH), 1746 (C=O), 1697 (C=O), 1693 (C=O); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 1.58 (9H, s, C(CH_3)$_3$), 3.23 (1H, dd, *J*=14.7, 2.3 Hz, C(6)*H*), 3.44 (1H, dd, *J*=14.7, 5.3 Hz, C(6)*H*`), 4.31 (1H, dd, *J*=15.8, 5.8 Hz, C(8)*H*), 4.45 - 4.52 (2H, m, C(8)*H*` and C(5)*H*), 6.76 (2H, d, *J*=8.2 Hz, Ar*H*), 6.88 (1H, d, *J*=2.2 Hz, Ar*H*), 6.92 (1H, t, *J*=7.5 Hz, Ar*H*), 7.04 (1H, t, *J*=7.5 Hz, Ar*H*), 7.36 (1H, d, *J*=8.0 Hz, Ar*H*), 7.44 (1H, d, *J*=8.0 Hz, Ar*H*), 7.74 (2H, d, *J*=8.2 Hz, Ar*H*), 8.72 (1H, br. t, *J*=5.7, 5.7 Hz, N(7)*H*), 10.97 (1H, br. d, *J*=2.2 Hz, indole N*H*), CO_2H not observed; ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 25.2 (C(CH_3)$_3$), 27.7 C(6), 45.3 (C(8)), 63.2 (C(5)), 84.0 (O*C*(CH_3)$_3$), 107.0, 111.3, 118.2, 118.4, 120.8, 123.8, 126.2, 127.6, 129.2, 135.8 (10 ArC), 150.2 (CO_2N), 167.2, 184.0 (2 C=O and C=N); *m/z* (ES^+) 463 ([M+H]$^+$, 10%); HRMS $C_{25}H_{27}N_4O_5$ requires: 463.1981, found: 463.1993.

4-(([1-(*tert*-butoxycarbonyl)-5-phenyl-4-oxo-4,5-dihydro-1*H*-imidazol-2-yl]amino)methyl)benzoic acid (28c)



To a solution of **27c** (0.25 g, 0.817 mmol) in ethanol (5 ml) was added 4-(aminomethyl)benzoic acid (0.124 g, 0.817 mmol), then stirred at reflux for 4 h. Cooled in a refrigerator and filtered to give a cream solid (30 mg). The filtrate was evaporated to give a white solid which was purified by column chromatography (hexane:ethyl acetate, 1:4) to give **28c** as a white solid (0.131 g, 0.319 mmol, 39%), mp 166-168 °C; ν_{max} (cm^{-1}) 3315 (NH/CO_2H), 1702 (C=O); ^{1}H NMR (500 MHz, methanol-d_4) δ ppm 1.24 (9H, s, C(CH_3)$_3$), 4.76 - 4.84 (2H, m, H(8)), 5.28 (1H, s, H(5)), 7.24 (2H, d, *J*=7.3 Hz, ArH), 7.32 - 7.43 (3H, m, ArH), 7.52 (2H, d, *J*=8.2 Hz, ArH), 8.03 (2H, d, *J*=8.5 Hz, ArH); ^{13}C NMR (125 MHz, methanol-d_4) δ ppm 27.9 (C(*C*H_3)$_3$), 47.5 (CH_2), 68.1 (CHPh), 86.1 (O*C*(CH_3)$_3$), 127.8, 128.5, 129.5, 129.9, 131.2, 131.4, 137.2, 143.9 (8 ArC), 151.7 (NC=OO), 169.4, 169.5 (C=N and C=O), 185.7 (C=O); *m/z* (ES^+) 410 ($[M+H]^+$, 8%); HRMS $C_{22}H_{24}N_3O_5^+$ requires: 410.1716, found: 410.1704.

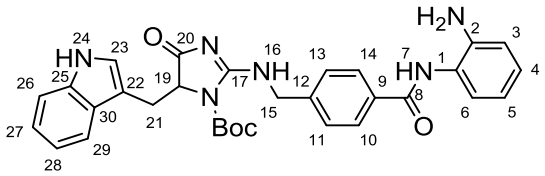
***tert*-butyl 2-[({4-[(2-aminophenyl)carbamoyl]phenyl}methyl)amino]-5-benzyl-4-oxo-4,5-dihydro-1*H*-imidazole-1-carboxylate (29a)**



Acid **28a** (230 mg, 0.543 mmol) was reacted according to the above procedure for **24a**, purification by column chromatography (2% methanol/dichloromethane) gave benzamide **29a** as a cream solid (154 mg, 0.299 mmol, 55%), mp 121-124 °C; ν_{max} (cm^{-1}) 3304 (NH), 1699 (C=O); ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 1.65 (9H, s, C(C*H*$_3$)$_3$), 3.29 - 3.39 (2H, m, H(21) and H(21)'), 3.92 (2H, br s, NH_2), 4.40 (1H, dd, *J*=15.4, 5.4 Hz, H(15)), 4.44 - 4.50 (1H, m, H(15)'), 4.73 (1H, dd, *J*=15.4, 7.2 Hz, H(19)), 6.81 - 6.89 (2H, m, H(5) and H(3)), 7.02 (2H, d, *J*=8.0 Hz, H(11 and 13)), 7.07 - 7.14 (3H, m, H(4) and H(23 and 27)), 7.17 - 7.40 (4H, m, H(6), H(24 and 26) and H(25)), 7.81 (2H, d, *J*=8.0 Hz, H(10 and 14)), 8.15 (1H, br s, H(16)), 8.41 (1H, br s, H(8)); ^{13}C NMR (125 MHz, chloroform-*d*) δ ppm 28.3 (C(*C*H_3)$_3$), 35.8 (C(21)), 46.2 (C(15)), 63.4 (C(19)), 85.6 (O*C*(CH_3)$_3$), 118.4 (C(3)), 119.8 (C(5)), 124.7 (C(1)), 125.4

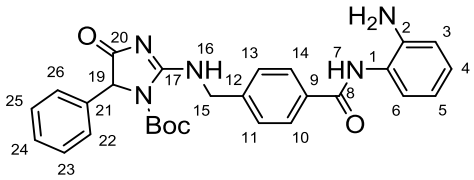
(C(4)), 127.3 (C(6)), 127.3 (C(25)), 127.6 (C(11 and 13)), 127.9 (C(10 and 14)), 128.6 (C(23 and 27)), 129.7 (C(24 and 26)), 133.6 (C(9)), 134.2 (C(22)), 140.6 (C(12)), 140.8 (C(2)), 151.1 (CO_2N), 165.5 (C(17)), 167.8 (C(8)), 184.1 (C(20)); *m/z* (ES^+) 514 ($[M+H]^+$, 10%), 536 ($[M+Na]^+$, 19%); HRMS $C_{29}H_{3Boc1}N_5O_4Na$, requires: 536.2274, found: 536.2277.

tert-butyl 2-[({4-[(2-aminophenyl)carbamoyl]phenyl}methyl)amino]-5-(1*H*-indol-3-ylmethyl)-4-oxo-4,5-dihydro-1*H*-imidazole-1-carboxylate (29b)



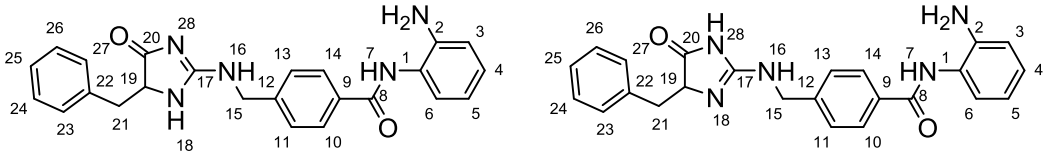
Acid **28b** (0.970 g, 2.10 mmol) was reacted according to the above procedure for **24a**, purification by column chromatography (6% methanol:chloroform) gave benzamide **29b** as a cream solid (0.398 g, 0.721 mmol, 34%), mp 185-186 °C; ν_{max} (cm^{-1}) 3288 (NH), 1732 (C=O), 1699 (C=O); ^{1}H NMR (500 MHz, chloroform-*d*) δ ppm 1.66 (9H, s, C(C*H*$_3$)$_3$), 3.30 (1H, dd, *J*=14.8, 2.4 Hz, H(21)), 3.49 (1H, dd, *J*=14.8, 3.7 Hz, H(21)'), 3.98 (2H, br s, NH_2), 4.12 (1H, dd, *J*=15.8, 5.2 Hz, H(15)), 4.41 - 4.45 (1H, m, H(15)'), 4.58 (1H, dd, *J*=15.8, 7.7 Hz, H(19)), 6.52 (2H, d, *J*=8.0 Hz, H(11 and 13)), 6.67 (1H, d, *J*=1.7 Hz, H(28)), 6.75 - 6.82 (2H, m, H(5) and H(3)), 6.99 (1H, t, *J*=7.5 Hz, H(4)), 7.03 - 7.12 (2H, m, H(23) and H(27)), 7.34 (1H, d, *J*=7.7 Hz, H(6)), 7.41 (1H, d, *J*=8.4 Hz, H(26)), 7.44 (1H, d, *J*=8.2 Hz, H(29)), 7.66 (2H, d, *J*=7.7 Hz, H(10 and 14)), 8.31 (1H, br s, H(16)), 8.71 (1H, br s, H(7)), 9.11 (1H, br s, H(24)); ^{13}C NMR (125 MHz, chloroform-*d*) δ ppm 25.7 (C(21)), 28.3 (C(*C*H$_3$)$_3$), 45.9 (C(15)), 64.2 (C(19)), 85.7 (O*C*(CH$_3$)$_3$), 107.1 (C(22)), 111.8 (C(26)), 117.8 (C(3)), 118.1 (C(5)), 119.3 (C(29)), 119.4 (C(28)), 121.7 (C(27)), 124.2 (C(23)), 124.6 (C(1)), 125.8 (C(4)), 126.5 (C(11 and 13)), 127.2 (C(6)), 127.9 (C(10 and 14)), 128.0 (C(30)), 133.2 (C(9)), 136.0 (C(25)), 140.3 (C(12)), 141.2 (C(2)), 151.0 (CO_2N), 166.0 (C(17)), 168.0 (C(8)), 185.4 (C(20)); *m/z* (ES^+) 553 ($[M+H]^+$, 15%), 575 ($[M+Na]^+$, 8%); HRMS $C_{31}H_{32}N_6O_4Na$, requires: 575.2383, found: 575.2389.

tert-butyl 2-[({4-[(2-aminophenyl)carbamoyl]phenyl}methyl)amino]-5-phenyl-4-oxo-4,5-dihydro-1*H*-imidazole-1-carboxylate (29c)



To a solution of benzoic acid **28c** (0.115 g, 0.281 mmol, 1 eq), BOP reagent (0.143 g, 0.323 mmol, 1.15 eq) and phenylene-1,2-diamine (0.061 g, 0.562 mmol, 2 eq) in DMF (3 ml) was added triethylamine (0.075 ml, 0.562 mmol, 2 eq) and stirred at rt for 4 h. Some DMF was evaporated then diluted with ethyl acetate (50 ml) and washed with sat. aq. lithium chloride (20 ml) and brine (2 × 20 ml). The crude product was purified by column chromatography on silica gel (ethyl acetate) to give the benzamide **29c** as a cream solid (0.139 g, 0.277 mmol, 98%), mp 178-180 °C; ν_{max} (cm^{-1}) 3318 (NH), 1736 (C=O), 1700 (C=O); ^{1}H NMR (500 MHz, chloroform-*d*) δ ppm 1.22 (9H, s, $C(CH_3)_3$), 4.02 (2H, br s, NH_2), 4.71 (1H, d, *J*=15.1 Hz, H(15)), 4.76 (1H, d, *J*=15.1 Hz, H(15)'), 5.10 (1H, s, H(19)), 6.78 (2H, d, *J*=7.3 Hz, H(11 and 13)), 7.03 (1H, t, *J*=7.6 Hz, H(5)), 7.17 (2H, d, *J*=7.3 Hz, H(22 and 26)), 7.24 (1H, d, *J*=7.6 Hz, H(3)), 7.27 - 7.39 (5H, m, H(23 and 25), H(24), H(4) and H(6))), 7.82 (2H, d, *J*=7.3 Hz, ArH, H(10 and 14)), 8.44 (1H, br s, H(16)), 8.79 (1H, br s, H(7)); ^{13}C NMR (125 MHz, chloroform-*d*) δ ppm 27.7 ($C(CH_3)_3$), 46.8 (C(15)), 66.7 (C(19)), 85.5 ($OC(CH_3)_3$), 118.5 (C(3)), 119.9 (C(5)), 124.6 (C(1)), 125.7 (C(4)), 126.5 (C(11 and 13)), 127.3 (C(6)), 128.0 (C(10 and 14)), 128.2 (C(23 and 25)), 128.6 (C(24)), 128.9 (C(22 and 26)), 133.8 (C(9)), 135.2 (C(22)), 140.4 (C(12)), 140.5 (C(2)), 150.8 (CO_2N), 165.8 (C(17)), 168.0 (C(8)), 183.1 (C(20)); *m/z* (ES^+) 522 ($[M+Na]^+$, 45%); HRMS $C_{28}H_{29}N_5O_4Na^+$ requires: 522.2117, found: 522.2103.

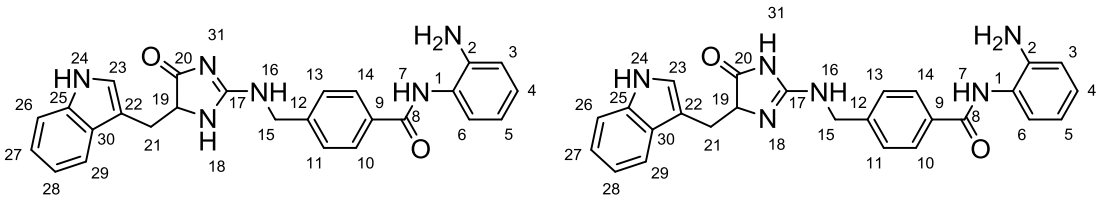
***N*-(2-aminophenyl)-4-({[5-benzyl-4-oxo-4,5-dihydro-1*H*-imidazol-2-yl]amino}methyl)benzamide (30a)**



To a solution of Boc-protected benzamide **29a** (100 mg, 0.195 mmol) in dichloromethane (1 ml) was added trifluoroacetic acid (1 ml). The resulting solution was stirred at rt for 3 h then solvents were evaporated, and the resulting oil was

azeotroped with toluene to give a pink solid. This solid was partitioned between sat. aq. sodium hydrogen carbonate (50 ml) and ethyl acetate (50 ml) then the organic layer was washed with brine, dried $MgSO_4$ and evaporated to give a yellow solid. Purification by column chromatography (10% methanol/dichloromethane) gave deprotected benzamide **30a** as a pale yellow solid (58 mg, 0.140 mmol, 72%), mp 157-160 °C; ν_{max} (cm^{-1}) 3197 (NH), 1699 (C=O); ^{1}H NMR (2:1 mix of tautomers) (600 MHz, DMSO-d_6) δ ppm 2.76 (1H, dd, *J*=13.6, 6.4 Hz, H(21)), 2.94 - 2.99 (0.33H, m, H(21)'), 3.03 (0.66H, dd, *J*=13.4, 3.2 Hz, H(21)'), 4.06 (1H, app. t, *J*=5.1 Hz, H(19)), 4.28 - 4.52 (2H, m, H(15) and H(15)'), 4.91 (2H, br s, N*H*$_2$), 6.60 (1H, t, *J*=7.3 Hz, H(5)), 6.79 (1H, d, *J*=7.9 Hz, H(3)), 6.97 (1H, t, *J*=7.5 Hz, H(4)), 7.10 - 7.31 (8H, m, Ar*H*), 7.63 (0.66H, br s, H(18)), 7.74 (0.66H, br s,H(16)), 7.89 (1.32H, d, *J*=7.2 Hz, H(10 and 14)), 7.95 (0.66H, br. d., *J*=7.2 Hz, H(10 and 14)), 8.23 (0.33H, br s, H(28)), 8.35 (0.33H, br s, H(16)), 9.64 (0.66H, br s, H(7)), 9.69 (0.33H, br s, H(7)); ^{13}C NMR (150 MHz, DMSO-d_6) δ ppm 37.2, 43.7, 44.7, 61.2, 116.1, 116.3, 123.3, 126.3, 126.5, 126.7, 126.7, 126.9, 127.8, 128.0, 128.1, 129.5, 129.6, 133.2, 133.3, 136.9, 137.3, 141.8, 142.8, 143.2, 165.1, 170.8, 171.5, 187.6, 188.1 (complicated mixture of tautomers); *m/z* (CI^+) 414 ($[M+H]^+$, 15%); HRMS $C_{24}H_{24}N_5O_2$, requires: 414.1930, found: 414.1935.

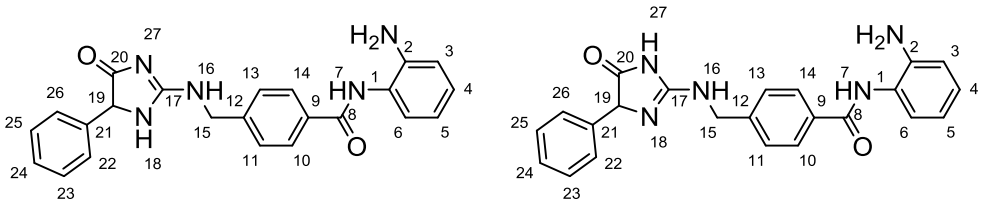
N-(2-aminophenyl)-4-({[5-(1*H*-indol-3-ylmethyl)-4-oxo-4,5-dihydro-1*H*-imidazol-2-yl]amino}methyl)benzamide (30b)



To a solution of Boc-protected benzamide **29b** (300 mg, 0.543 mmol) in dichloromethane (3 ml) was added trifluoroacetic acid (1.5 ml). The resulting solution was stirred at rt for 3 h then solvents were evaporated, and the resulting oil was azeotroped with toluene to give an orange solid. This solid was partitioned between sat. aq. sodium hydrogen carbonate (100 ml) and ethyl acetate (150 ml) then the organic layer was washed with brine, dried $MgSO_4$ and evaporated to give **30b** as a pale yellow solid (240 mg, 0.530 mmol, 98%), mp 205-207 °C. Recrystallisation of 50 mg from dichloromethane:methanol gave 30 mg of **30b** as a cream solid, mp 214-215 °C; ν_{max} (cm^{-1}) 3216 (NH), 1689 (C=O), 1662 (C=O); ^{1}H NMR (2:1 mixture of tautomers) (400 MHz, DMSO-d_6) δ ppm 2.86 (1H, dd, *J*=14.8, 7.0 Hz, H(21)), 3.16 (1H, br. dd, *J*=14.8, 5.3 Hz, H(21)), 4.03 - 4.15 (1H, m, H(19)), 4.34 - 4.50 (2H, m, H(15) and H(15)'), 4.91

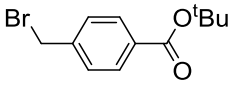
(2H, br s, NH_2), 6.61 (1H, t, *J*=7.4 Hz, H(5)), 6.79 (1H, d, *J*=7.5 Hz, H(3)), 6.90 - 7.39 (8H, m, ArH), 7.57 (1.66H, br s, 1 × ArH and H(18)), 7.68 (0.66H, br s, H(16)), 7.86 - 7.96 (2H, m, H(10 and 14)), 8.18 (0.33H, br s, H(31)), 8.33 (1H, br s, H(16)), 9.62 (1H, br s, H(7)), 10.83 - 10.92 (1H, m, H(24)); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 27.4, 43.7, 44.7, 48.6, 54.9, 61.1, 76.6, 106.8, 109.9, 111.2, 116.1, 116.3, 118.2, 118.6, 120.8, 123.3, 126.5, 126.6, 127.5, 127.8, 133.2, 136.0, 142.8, 143.1, 165.1, 171.7, 178.1, 184.9, 188.6 (complicated mixture of tautomers); *m/z* (ES^+) 453 ($[M+H]^+$, 100%); HRMS $C_{26}H_{25}N_6O_2$, requires: 453.2039, found: 453.2023.

_N_-(2-aminophenyl)-4-(([4-oxo-5-phenyl-4,5-dihydro-1_H_-imidazol-2-yl]amino)methyl)benzamide (30c)



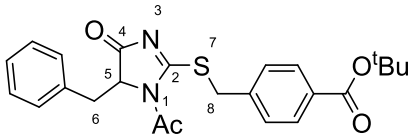
To a solution of Boc protected compound **29c** (0.135 g, 0.270 mmol) in dichloromethane (1.5 ml) was added trifluoroacetic acid (0.75 ml) and stirred at rt for 3 h. The solution was evaporated and azeotroped with toluene × 4. Then dissolved in methanol (10 ml) and sat. aq. sodium hydrogen carbonate (10 ml) was added and the methanol was evaporated, diluted with 10 ml water and the suspension was sonicated to break up big particles. Then filtered, washed with water and diethyl ether and dried under vacuum to give **30c** as a cream solid (0.075 g, 0.188 mmol, 70%), mp 221-223 °C; ν_{max} (cm^{-1}) 3263 (NH), 3062 (NH), 1706 (C=O), 1654 (C=O); (A mixture of NH tautomers was observed by NMR) ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 4.47 - 4.63 (2H, m, H(15) and H(15)'), 4.85 (1H, s, H(19)), 4.89 (2H, br s, NH_2), 6.59 (1H, t, *J*=7.1 Hz, H(5)), 6.77 (1H, d, *J*=7.9 Hz, H(3)), 6.96 (1H, t, *J*=7.1 Hz, H(4)), 7.12 - 7.20 (2H, m, ArH), 7.20 - 7.30 (2H, m, ArH), 7.30 - 7.39 (2H, m, ArH), 7.41 - 7.49 (2H, m, ArH), 7.92 - 8.02 (2H, m, H(10 and 14)), 8.12 - 8.24 (1H, m, major tautomer H(16) and H(18)), 8.72 (1H, br s, minor tautomer H(16) and H(27)), 9.63 (1H, br s, H(7)); ^{13}C NMR (150 MHz, DMSO-d_6) δ ppm 43.4, 43.8, 45.0, 45.1, 63.8, 116.2, 116.5, 123.2, 123.3, 125.6, 125.8, 126.5, 126.6, 126.7, 127.0, 127.1, 127.2, 127.4, 127.5, 127.5, 127.7, 128.0, 128.2, 128.3, 128.4, 128.6, 133.3, 133.4, 137.7, 141.6, 142.7, 143.0, 143.1, 165.2, 171.7, 186.7 (complicated mixture of tautomers); *m/z* (ES^+) 400 ($[M+H]^+$, 100%); HRMS $C_{23}H_{22}N_5O_2^+$ requires: 400.1774, found: 400.1755.

***tert*-butyl 4-(bromomethyl)benzoate (31)**[146]



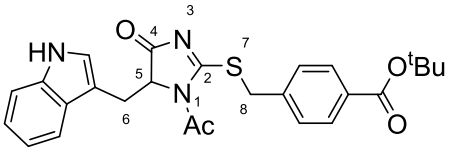
4-(bromomethyl)benzoic acid (1.00 g, 4.65 mmol) was dissolved in $SOCl_2$ (2.5 ml) and then stirred at reflux under nitrogen for 3 h. Then excess $SOCl_2$ was evaporated to give a cream solid. This solid was dissolved in anhydrous diethyl ether (70 ml) and to this stirring solution under nitrogen was added potassium *tert*-butoxide (0.522 g, 4.65 mmol) portionwise over 1.5 h and then stirred for a further 1.5 h at rt under nitrogen. The mixture was filtered washing with diethyl ether and then the filtrate was evaporated to give **31** as a white solid (0.802 g, 2.96 mmol, 64%), mp 55-56 °C (lit.[146] 58-59); ^{1}H NMR (500 MHz, chloroform-*d*) δ ppm 1.59 (9H, s, C(C*H*$_3$)$_3$), 4.49 (2H, s, C*H*$_2$Br), 7.43 (2H, d, *J*=8.2 Hz, Ar*H*), 7.96 (2H, d, *J*=8.2 Hz, Ar*H*); ^{13}C NMR (125 MHz, chloroform-*d*) δ ppm 28.2 (C(*C*H$_3$)$_3$), 32.5 (*C*H$_2$Br), 81.3 (O*C*(CH$_3$)$_3$), 128.9 (4-aryl), 130.0 (2,6-aryl), 132.0 (3,5-aryl), 142.1 (1-aryl), 165.2 (C=O); *m/z* (EI$^+$) 270 (M$^+$, 2%), 135 ([M-Br-C(CH$_3$)$_3$+H$^+$]$^+$, 100%).

***tert*-butyl 4-({[1-acetyl-5-benzyl-4-oxo-4,5-dihydro-1*H*-imidazol-2-yl]sulfanyl}methyl)benzoate (32a)**



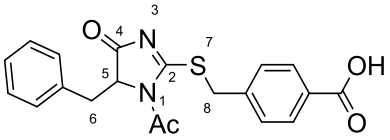
To a solution of thiohydantoin **(*S*)-21a** (0.298 g, 1.20 mmol) in acetonitrile (20 ml) was added *tert*-butyl 4-(bromomethyl)benzoate **31** (0.358 g, 1.32 mmol) and triethylamine (0.184 ml, 1.32 mmol). The resulting solution was stirred at 40 °C for 5.5 hours, then evaporated to give a cream solid. Purification by column chromatography (ethyl acetate:hexane, 1:1) gave **32a** as a white solid (0.385 g, 0.877 mmol, 73%), mp 143-144 °C; ν_{max} (cm^{-1}) 3447 (NH), 1702 (C=O), 1662 (C=O); ^{1}H NMR (500 MHz, DMSO-*d*$_6$) δ ppm 1.54 (9H, s, C(C*H*$_3$)$_3$), 2.41 (3H, s, COC*H*$_3$), 3.16 (1H, dd, *J*=14.3, 2.6 Hz, C(6)*H*), 3.36 (1H, dd, *J*=14.3, 5.8 Hz, C(6)*H*`), 4.15 (2H, d, *J*=14.0 Hz, C(8)*H*), 4.28 (1H, d, *J*=14.0 Hz, C(8)*H*`), 4.98 (2H, dd, *J*=5.8, 2.6 Hz, C(5)*H*), 6.96 - 7.01 (2H, m, Ar*H*), 7.17 - 7.21 (3H, m, Ar*H*), 7.22 (2H, d, *J*=8.2 Hz, Ar*H*), 7.75 (2H, d, *J*=8.2 Hz, 2,6-aryl); ^{13}C NMR (125 MHz, DMSO-*d*$_6$) δ ppm 23.6 (CO*C*H$_3$), 27.8 (C(*C*H$_3$)$_3$), 35.5 (C(6)), 36.0 (C(8)), 64.2 (C(5)), 80.7 (O*C*(CH$_3$)$_3$), 127.0, 128.2, 128.9, 129.1, 129.4, 130.1, 133.8, 141.8 (8 ArC), 164.7, 168.6, 184.1, 184.5 (3 C=O and C=N); *m/z* (EI$^+$) 438 (M$^+$, 67%); HRMS $C_{24}H_{26}O_4N_2S$ requires: 438.1608, found: 438.1612.

tert-butyl 4-({[1-acetyl-5-(1*H*-indol-3-ylmethyl)-4-oxo-4,5-dihydro-1*H*-imidazol-2-yl]sulfanyl}methyl)benzoate (32b)



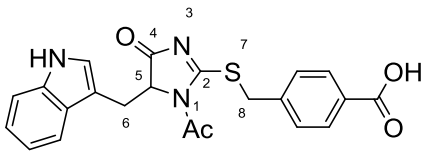
To a solution of thiohydantoin **(*S*)-21b** (0.752 g, 2.62 mmol) in acetonitrile (45 ml) was added *tert*-butyl 4-(bromomethyl)benzoate **31** (0.780 g, 2.88 mmol) and triethylamine (0.40 ml, 2.88 mmol). The resulting solution was stirred at 40 °C for 2.5 hours, then evaporated to give a cream solid. Purification by column chromatography (ethyl acetate:hexane, 1:1) gave **32b** as a white solid (0.708 g, 1.48 mmol, 56%), mp 183-185 °C; ν_{max} (cm^{-1}) 3320 (NH), 1738 (C=O), 1690 (C=O); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 1.55 (9H, s, C(C*H*$_3$)$_3$), 2.43 (3H, s, COC*H*$_3$), 3.38 (1H, dd, *J*=15.1, 2.3 Hz, C(6)*H*), 3.50 (3H, dd, *J*=15.1, 5.5 Hz, C(6)*H*`), 4.09 (1H, d, *J*=14.0 Hz, C(8)*H*), 4.30 (1H, d, *J*=14.0 Hz, C(8)*H*`), 4.97 (1H, dd, *J*=5.6, 2.3 Hz, C(5)*H*), 6.91 - 6.96 (1H, m, Ar*H*), 6.99 (1H, d, *J*=2.4 Hz, Ar*H*), 7.02 - 7.08 (3H, m, Ar*H*), 7.32 (1H, d, *J*=8.0 Hz, Ar*H*), 7.39 (1H, d, *J*=7.9 Hz, Ar*H*), 7.69 (2H, d, *J*=8.4 Hz, Ar*H*), 10.96 (1H, br. d, *J*=2.4 Hz, N*H*); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 23.6 (CO*C*H$_3$), 26.6 (C(6)), 27.8 (C(*C*H$_3$)$_3$), 35.3 (C(8)), 64.3 (C(5)), 80.6 (O*C*(CH$_3$)$_3$), 106.1, 111.3, 118.4, 118.6, 120.9, 124.4, 127.1, 128.7, 129.1, 130.1, 135.8, 141.8 (12 ArC), 164.7, 168.6, 184.1, 185.1 (3 C=O and C=N); *m/z* (ES^-) 476 (M^-, 100%); HRMS $C_{26}H_{26}O_4N_3S$ requires: 476.1634, found: 476.1644.

4-({[1-acetyl-5-benzyl-4-oxo-4,5-dihydro-1*H*-imidazol-2-yl]sulfanyl}methyl)benzoic acid (33a)



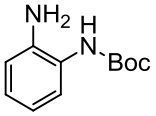
To a solution of **32a** (0.318 g, 0.725 mmol) in dichloromethane (3 ml) was added trifluoroacetic acid (3 ml) and the solution stirred at rt for 2 h. The solvent was evaporated and then azeotroped with toluene to give **33a** as a white solid (0.272 g, 0.711 mmol) which contained an unidentified impurity (~30%) however TLC indicated that column chromatography was unsuitable for the separation of the impurity. Therefore the material was used impure.

4-({[1-acetyl-5-(1*H*-indol-3-ylmethyl)-4-oxo-4,5-dihydro-1*H*-imidazol-2-yl]sulfanyl}methyl)benzoic acid (33b)



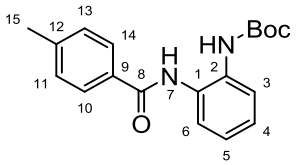
To a solution of **32b** (0.689 g, 1.44 mmol) in dichloromethane (6 ml) was added trifluoroacetic acid (3 ml) and the solution stirred at rt for 45 min. The solvent was evaporated and then azeotroped with toluene to give a pale yellow solid which was triturated with diethyl ether and filtered to give **33b** as a white solid (0.498 g, 1.18 mmol, 82%), mp 139-141 °C; ν_{max} (cm^{-1}) 3452 (NH), 1720 (C=O), 1698 (C=O); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 2.42 (3H, s, COCH_3), 3.34 - 3.41 (1H, m, C(6)*H*), 3.50 (1H, dd, *J*=15.4, 5.6 Hz, C(6)*H*`), 4.13 (1H, d, *J*=14.0 Hz, C(8)*H*), 4.27 (1H, d, *J*=14.0 Hz, C(8)*H*`), 4.97 (1H, dd, *J*=5.5, 2.8 Hz, C(5)*H*), 6.93 (1H, t, *J*=7.2 Hz, Ar*H*), 6.99 (1H, d, *J*=2.2 Hz, Ar*H*), 7.04 (1H, t, *J*=7.2 Hz, Ar*H*), 7.12 (2H, d, *J*=8.2 Hz, Ar*H*), 7.31 (1H, d, *J*=8.0 Hz, Ar*H*), 7.39 (1H, d, *J*=7.9 Hz, Ar*H*), 7.77 (2H, d, *J*=8.2 Hz, Ar*H*), 10.96 (1H, br s, N*H*), 12.90 (1H, br s, CO_2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 23.6 (CO*C*H_3), 26.6 (C(6)), 35.4 (C(8)), 64.3 (C(5)), 106.2, 111.3, 118.3, 118.6, 120.9, 124.3, 127.1, 128.8, 129.4, 129.6, 135.8, 141.7 (12 ArC), 167.0, 168.6, 184.0, 185.2 (3 C=O and C=N); *m/z* (ES^+) 422 ($[M+H]^+$, 32%), 444 ($[M+Na]^+$, 25%); HRMS $C_{22}H_{19}O_4N_3S$ requires: 444.0994, found: 444.1003.

***tert*-butyl (2-aminophenyl)carbamate (39)**[147]



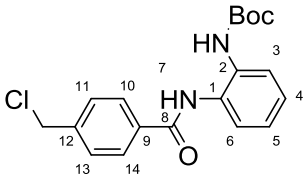
To a solution of phenylene-1,2-diamine (1.63 g, 15.1 mmol) in THF (25 ml) was added a solution of di-*tert*-butyl dicarbonate in THF (5 ml) dropwise at rt. Then stirred for a further 2 h at rt and the solvent was evaporated. Purification by column chromatography (hexane:ethyl acetate, 4:1) gave **39** as a white solid (2.56 g, 12.3 mmol, 81%), mp 112-114 °C (lit. 114 °C)[148]; ^{1}H NMR (500 MHz, chloroform-*d*) δ ppm 1.51 (9H, s, C(CH_3)$_3$), 3.70 (2H, br s, NH_2), 6.29 (1H, br s, N*H*), 6.70 - 6.83 (2H, m, Ar*H*), 6.99 (1H, td, *J*=7.6, 1.3 Hz, Ar*H*), 7.23 - 7.32 (1H, m, Ar*H*); ^{13}C NMR (125 MHz, chloroform-*d*) δ ppm 28.4 (C(*C*H_3)$_3$), 80.6 (O*C*(CH$_3$)$_3$), 117.7, 119.7, 124.9, 126.2, 140.0 (5 ArC), 153.9 (C=O).

tert-butyl (2-(4-methylbenzamido)phenyl)carbamate (40)



To a solution of aryl amine **39** (200 mg, 0.96 mmol) and triethylamine (0.268 mL, 1.92 mmol) in dichloromethane (3 mL) was added *para*-toluoyl chloride (0.127 mL, 0.96 mmol) then the mixture was stirred at rt for 4.5 h under a nitrogen atmosphere. Diluted with dichloromethane (20 mL) and washed with sodium hydrogen carbonate (sat. aq., 2 × 20 mL), brine (20 mL) and dried $MgSO_4$ then evaporated. The resulting beige solid was triturated with hexane and filtered to give **40** as a white solid (0.267 g, 0.817 mmol, 85%), mp 124-126 °C; ν_{max} (cm^{-1}) 3246 (NH), 1687 (C=O), 1651 (C=O); ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 1.53 (9H, s, $C(CH_3)_3$), 2.44 (3H, s, CH_3), 6.98 (1H, br s, BocNH), 7.09 - 7.23 (2H, m, ArH), 7.24 - 7.35 (3H, m, H(11 and 13) and ArH), 7.73 (1H, d, *J*=7.8 Hz, ArH), 7.88 (2H, d, *J*=8.0 Hz, H(10 and 14)), 9.14 (1H, br s, H(7)); ^{13}C NMR (125 MHz, chloroform-*d*) δ ppm 21.6 (CH_3), 28.4 ($C(CH_3)_3$), 81.3 ($OC(CH_3)_3$), 124.6 (C(3)), 125.8 (C(5)), 125.9 (C(4)), 125.9 (C(6)), 127.5 (C(10 and 14)), 129.3 (C(11 and 13)), 130.1 (C(2)), 131.0 (C(9)), 131.5 (C(1)), 142.5 (C(12)), 154.7 (NCOO), 165.8 (C(8)); *m/z* (ESI^+) 327 ($[M+H]^+$, 100%); HRMS $C_{19}H_{23}N_2O_3^+$ requires: 327.1703, found: 327.1710.

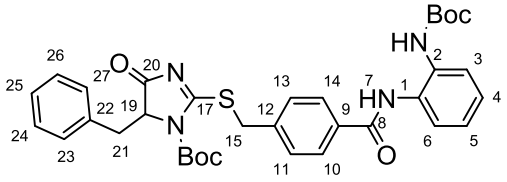
tert-butyl (2-([4-(chloromethyl)benzoyl]amino)phenyl)carbamate (43)



To a solution of **39** (2.55 g, 12.2 mmol, 1.0 eq) in dichloromethane (50 ml) cooled to -10 °C, was added triethylamine (1.86 ml, 13.4 mmol, 1.1 eq) then a solution of 4-(chloromethyl)benzoyl chloride (2.54 g, 13.4 mmol, 1.1 eq) in dichloromethane (20 ml)dropwise. The stirred solution was allowed to warm to rt and stirred for 1 h. Then evaporated and suspended/dissolved in diethyl ether and filtered. The solid was dissolved in dichloromethane (200 ml) and washed successively with sat. aq. ammonium chloride (200 ml), sat. aq. sodium hydrogen carbonate (200 ml), brine, then dried over $MgSO_4$ and evaporated to give **43** as a white powdery solid (4.02 g, 11.2 mmol, 91%), mp 172-173 °C; ν_{max} (cm^{-1}) 3277 (NH), 1682 (C=O), 1655 (C=O); ^{1}H NMR (500 MHz, chloroform-*d*) δ ppm 1.51 (9H, s, $C(CH_3)_3$), 4.63 (2H, s, CH_2Cl), 6.83

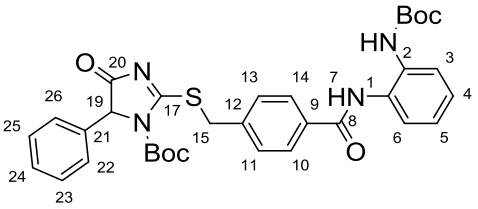
(1H, br s, BocNH), 7.14 (1H, t, *J*=7.6 Hz, H(5)), 7.21 (2H, m, H(4) and H(6)), 7.48 (2H, d, *J*=8.2 Hz, H(11 and 13)), 7.77 (1H, d, *J*=7.9 Hz, H(3)), 7.96 (2H, d, *J*=8.2 Hz, H(10 and 14), 9.27 (1H, br s, H(7)); ^{13}C NMR (125 MHz, chloroform-*d*) δ ppm 28.4 (C(*C*H$_3$)$_3$), 45.5 (CH_2Cl), 81.6 (O*C*(CH$_3$)$_3$), 124.6 (C(3)), 125.9 (C(6)), 126.1 (C(4)), 126.1 (C(5)), 127.9 (C(10 and 14)), 128.8 (C(11 and 13)), 129.9 (C(9)), 130.9 (C(2)), 134.3 (C(1)), 141.3 (C(12)), 154.8 (CO_2N), 165.1 (C(8)); *m/z* (ES$^+$) 383 ([M+Na]$^+$, 90%); HRMS $C_{19}H_{21}N_2O_3ClNa^+$ requires: 383.1138, found: 383.1136.

4-({[1-(*tert*-butoxycarbonyl)-5-benzyl-4-oxo-4,5-dihydro-1*H*-imidazol-2-yl]sulfanyl}methyl)-*N*-(2-aminophenyl)benzamide (44)



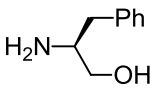
To a solution of the benzyl chloride **43** (0.7 g, 1.94 mmol, 1.1 eq) in acetone (15 ml) was added sodium iodide (0.582 g, 3.88 mmol, 2.2 eq) then stirred at reflux for 2 h (the round bottomed flask, oil bath and condenser were wrapped in foil to keep the benzyl iodide in the dark). After 2 h the mixture was cooled to rt and a solution of thiohydantoin **44** (0.539 g, 1.76 mmol, 1.0 eq) in dichloromethane (5 ml) was added followed by potassium carbonate (0.365 g, 2.64 mmol, 1.5 eq). The resulting mixture was stirred at rt for 20 h (in the dark). The mixture was then filtered then evaporated. The residue was purified by column chromatography (hexane:ethyl acetate, 2:1 to 1:1) to give **44** as a white solid (0.137 g, 0.217 mmol, 12%), mp 104-106 °C; ν_{max} (cm^{-1}) 3287 (NH), 1722 (C=O); ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 1.53 (9H, s, C(CH$_3$)$_3$)), 1.64 (9H, s, C(CH$_3$)$_3$)), 3.35 (1H, dd, *J*=13.8, 2.5 Hz, H(21)), 3.45 (1H, dd, *J*=13.8, 5.8 Hz, H(21)'), 4.22 (1H, d, *J*=13.8 Hz, H(15)), 4.37 (1H, d, *J*=13.8 Hz, H(15)'), 4.54 - 4.59 (1H, m, H(19)), 6.87 (1H, br s, BocNH), 7.04 - 7.08 (2H, m, ArH), 7.16 - 7.31 (8H, m, ArH), 7.80 (1H, d, *J*=7.5 Hz, H(6)), 7.86 (2H, d, *J*=8.3 Hz, H(10 and 14)), 9.23 (1H, br s, H(7)); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 28.1 (C(*C*H$_3$)$_3$), 28.3 (C(*C*H$_3$)$_3$), 36.0 (C(15)), 36.7 (C(21)), 64.5 (C(19)), 81.4 (O*C*(CH$_3$)$_3$), 85.9 (O*C*(CH$_3$)$_3$), 124.5 (C(3)), 125.7 (C(6)), 125.9 (C(4)), 126.0 (C(5)), 127.5 (C(24)), 127.7 (11 and 13), 128.5 (C(22 and 26)), 129.3 (10 and 14), 129.3 (23 and 25), 130.0 (C(9)), 130.8 (C(2)), 133.4 (C(1) and C(21)), 139.8 (C(12)), 148.3 (CO_2 CO_2N), 154.6 (CO_2N), 165.1 (C(17)), 184.6 (C(8)); *m/z* (ES$^+$) 653 ([M+Na]$^+$, 100%); HRMS $C_{34}H_{38}N_4O_6SNa^+$ requires: 653.2410, found: 653.2408.

4-({[1-(*tert*-butoxycarbonyl)-5-phenyl-4-oxo-4,5-dihydro-1*H*-imidazol-2-yl]sulfanyl}methyl)-*N*-(2-aminophenyl)benzamide (45)



To a solution of the benzyl chloride **43** (253 mg, 0.70 mmol, 1.0 eq) in dichloromethane (7 ml) was added thiohydantoin **26c**, potassium carbonate (290 mg, 2.1 mmol, 3.0 eq), sodium iodide (105 mg, 0.7 mmol, 1.0 eq) and acetone (3 ml), the mixture was then heated to reflux for 25 h. Then evaporated, the residue was portioned between ethyl acetate and water (10 ml each) then separated, then organic layer was washed with brine, dried $MgSO_4$ and concentrated to give an orange foam which was purified by column chromatography (dichloromethane:ethyl acetate, 4:1) to give **45** as a white solid (130 mg, 0.21 mmol, 30%), mp 133-135 °C; ν_{max} (cm^{-1}) 3296 (NH), 1724 (C=O); ^{1}H NMR (500 MHz, chloroform-*d*) δ ppm 1.25 (9H, s, $C(CH_3)_3$), 1.50 (9H, s, $C(CH_3)_3$), 4.54 (1H, d, *J*=13.6 Hz, H(15)), 4.59 (1H, d, *J*=13.6 Hz, H(15)'), 5.18 (1H, s, H(19)), 6.90 (1H, br s, BocNH), 7.11 - 7.25 (5H, H(5), H(3), H(22 and 26) and H(24)), 7.31 - 7.40 (3H, m, H(23 and 25) and H(4)), 7.54 (2H, d, *J*=8.2 Hz, H(11 and 13)), 7.75 (1H, d, *J*=7.6 Hz, H(6)), 7.93 (2H, d, *J*=8.2 Hz, H(10 and 14)), 9.26 (1H, br s, H(7)); ^{13}C NMR (125 MHz, chloroform-*d*) δ ppm 27.7 ($C(CH_3)_3$), 28.4 ($C(CH_3)_3$), 37.4 (C(15)), 67.5 (C(19)), 81.5 ($OC(CH_3)_3$), 85.6 ($OC(CH_3)_3$), 124.6 (C(3)), 125.9 (C(6)), 126.0 (C(4)), 126.1 (C(5)), 126.4 (C(10 and 14)), 128.0 (C(11 and 13)), 128.9 (C(24)), 129.1 (C(23 and 25)), 129.7 (C(22 and 26)), 130.1 (C(9)), 130.9 (C(2)), 133.9 (C(1)), 134.3 (C(21)), 139.6 (C(12)), 148.4 (CO_2N), 154.7 (CO_2N), 165.2 (C(17)), 183.0 (C(20)), 185.2 (C(8)); *m/z* (ES^+) 639 ($[M+Na]^+$, 100%); HRMS $C_{33}H_{36}N_4O_6SNa^+$ requires: 639.2253, found: 639.2264.

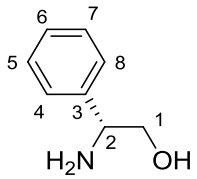
L-Phenylalinol (46a)[149]



A 2-neck flask (oven-dried) was fitted with a magnetic stirrer and a reflux condenser and then $NaBH_4$ (1.36 g, 36.1 mmol, 2.4 eq) and L-Phenylalanine (2.48 g, 15.0 mmol, 1.0 eq) were added, followed by dry THF (40 ml). Then a dropping funnel was fitted and the mixture cooled to 0 °C in an ice bath. A solution of iodine (3.80 g,

15.0 mmol, 1.0 eq) in THF (10 ml) was added dropwise over 30 min, vigorous gas evolution was observed, after gas evolution had stopped the mixture was heated to reflux for 18 h. Then cooled to rt and methanol added slowly until solution became clear. Stirred for 30 min and then the solvent was evaporated. The resulting white paste was dissolved in 30 ml of 20% aq. potassium hydroxide and stirred for 4 h then extracted with dichloromethane (3 × 30 ml). The combined organics were washed with brine, and dried Na_2SO_4 and evaporated to give a crystalline solid which was recrystallized from toluene to give **46a** as a white solid (1.19 g, 7.87 mmol, 52%), mp 88-90 °C (lit. 90-92 °C)[149]; ^{1}H NMR (500 MHz, chloroform-*d*) δ ppm 2.04 (3H, br s, NH_2 and OH), 2.52 (1H, dd, *J*=13.5, 8.7 Hz, C*H*H`Ph), 2.79 (1H, dd, *J*=13.5, 5.0 Hz, CH*H*`Ph), 3.07 - 3.17 (1H, m, NCH), 3.39 (1H, dd, *J*=10.7, 7.3 Hz, C*H*H`OH), 3.63 (1H, dd, *J*=10.7, 3.8 Hz, CH*H*`OH), 7.16 - 7.27 (3H, m, ArH), 7.28 - 7.34 (2H, m, ArH); ^{13}C NMR (125 MHz, chloroform-*d*) δ ppm 40.9 (CH_2Ph), 54.3 (CHN), 66.3 (CwaterH), 126.5 (*p*-Ph), 128.7 (*o*-Ph), 129.3 (*m*-Ph), 138.7 (*i*-Ph).

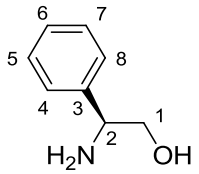
(*2R*)-2-amino-2-phenylethan-1-ol ((*R*)-46b)



A 3-neck flask (oven-dried) was fitted with a magnetic stirrer and a reflux condenser and then $NaBH_4$ (0.756 g, 20 mmol, 2.0 eq) was added followed by dry THF (20 ml). Then a dropping funnel was fitted and the mixture cooled to 0 °C in an ice bath. A solution of iodine (2.54 g, 10.0 mmol, 1.0 eq) in THF (10 ml) was added dropwise over 20 min, vigorous gas evolution was observed, after gas evolution had stopped D-phenylglycine (1.51 g, 10.0 mmol, 1.0 eq) was added and the mixture was heated to reflux for 16.5 h. Then cooled to rt and methanol added slowly until solution became clear. Stirred for 30 min and then the solvent was evaporated. The resulting white paste was dissolved in 30 ml of 20% aq. potassium hydroxide and stirred for 4 h then extracted with dichloromethane (3 × 30 ml). The combined organics were washed with brine (20 mL then 75 mL), dried Na_2SO_4 and then evaporated to give a white semi-solid which was recrystallized from toluene to give **(*R*)-46b** as a white crystalline solid (0.68 g, 4.78 mmol, 48%), mp 73-75 °C (lit. 69-71 °C)[149]; ^{1}H NMR (500 MHz, chloroform-*d*) δ ppm 2.38 (3H, br s, NH_2 and OH), 3.58 (1H, dd, *J*=10.8, 8.5 Hz, H(1)), 3.75 (1H, dd, *J*=10.8, 4.2 Hz, H(1)`), 4.06 (1H, dd, *J*=8.2, 4.2 Hz, H(2)), 7.23 - 7.45 (5H, m, ArH);

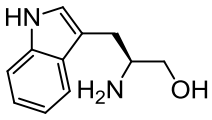
^{13}C NMR (125 MHz, chloroform-*d*) δ ppm 57.8 (C(1)), 68.4 (C(2)), 126.9 (C(4 and 8)), 127.9 (C(6)), 129.0 (C(5 and 7)), 143.0 (C(3)).

(*2S*)-2-amino-2-phenylethan-1-ol ((*S*)-46b)[149]



A 3-neck flask (oven-dried) was fitted with a magnetic stirrer and a reflux condenser and then $NaBH_4$ (0.756 g, 20 mmol, 2.0 eq) was added followed by dry THF (20 ml). Then a dropping funnel was fitted and the mixture cooled to 0 °C in an ice bath. A solution of iodine (2.54 g, 10.0 mmol, 1.0 eq) in THF (10 ml) was added dropwise over 20 min, vigorous gas evolution was observed, after gas evolution had stopped L-phenylglycine (1.51 g, 10.0 mmol, 1.0 eq) was added and the mixture was heated to reflux for 16 h. Then cooled to rt and methanol added slowly until solution became clear. Stirred for 30 min and then the solvent was evaporated. The resulting white paste was dissolved in 30 ml of 20% aq. potassium hydroxide and stirred for 4 h then extracted with dichloromethane (3 × 30 ml). The combined organics were washed with brine (20 mL then 75 mL), dried Na_2SO_4 and then evaporated to give a white semi-solid which was recrystallized from toluene to give **(*S*)-46b** as a white crystalline solid (0.68 g, 4.69 mmol, 50%), mp 76-77 °C (lit. 76-78 °C)[150]; ^{1}H NMR (500 MHz, chloroform-*d*) δ ppm 2.25 (3H, br s, NH_2 and OH), 3.55 (1H, dd, *J*=10.9, 8.3 Hz, H(1)), 3.73 (1H, dd, *J*=10.9, 4.3 Hz, H(1)`), 4.04 (1H, dd, *J*=8.3, 4.3 Hz, H(2)), 7.24 - 7.29 (1H, m, H(6)), 7.30 - 7.38 (4H, m, H(4 and 8) and H(5 and7)); ^{13}C NMR (125 MHz, chloroform-*d*) δ ppm 57.4 (C(1)), 68.1 (C(2)), 126.6 (C(4 and 8)), 127.6 (C(6)), 128.7 (C(5 and 7)), 142.7 (C(3)).

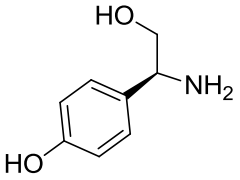
(2*S*)-2-amino-3-(1*H*-indol-3-yl)propan-1-ol (46c)[151]



A 3-neck flask (oven-dried) was fitted with a magnetic stirrer and a reflux condenser and then $NaBH_4$ (0.756 g, 20.0 mmol, 2.0 eq) and L-tryptophan (2.04 g, 10.0 mmol, 1.0 eq) were added, followed by dry THF (20 ml). Then a dropping funnel was fitted and the mixture cooled to 0 °C in an ice bath. A solution of iodine (2.54 g, 10.0

mmol, 1.0 eq) in THF (10 ml) was added dropwise over 20 min, vigorous gas evolution was observed, after gas evolution had stopped the mixture was heated to reflux for 17 h. Then cooled to rt and methanol added slowly until solution became clear. Stirred for 30 min and then the solvent was evaporated. The resulting white paste was dissolved in 20 ml of 20% aq. potassium hydroxide and stirred for 20 h then neutralised slightly with hydrochloric acid (2M aq. to ~ pH 11) and extracted with dichloromethane (3 × 20 ml) then 20% ethanol/chloroform (4 × 25 ml). The combined organics were washed with brine, and dried Na_2SO_4 and evaporated to give **46c** as a yellow oil (1.38 g, 7.27 mmol, 73%); ν_{max} (cm^{-1}) 3401 (OH), 3251 (NH); ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 1.73 (3H, br s, NH_2 and OH), 2.74 (1H, dd, *J*=14.4, 8.4 Hz, H(10)), 2.97 (1H, dd, *J*=14.4, 5.1 Hz, H(10)`), 3.25 - 3.33 (1H, m, H(11)), 3.46 (1H, dd, *J*=10.5, 7.2 Hz, H(12)), 3.72 (1H, dd, *J*=10.5, 4.0 Hz, H(12)), 7.08 (1H, d, *J*=2.0 Hz, H(2)), 7.15 (1H, app. t, *J*=7.3 Hz, H(6)), 7.24 (1H, app. t, *J*=7.2 Hz, H(7)), 7.40 (1H, d, *J*=8.0 Hz, H(5)), 7.64 (1H, d, *J*=8.0 Hz, H(8)), 8.09 (1H, br s, H(1)); ^{13}C NMR (150 MHz, chloroform-*d*) δ ppm 30.5 (C(10)), 53.0 (C(11)), 66.9 (C(12)), 111.3 (C(8)), 112.8 (C(3)), 119.0 (C(7)), 119.6 (C(5)), 122.3 (C(6)), 122.7 (C(2)), 127.7 (C(4)), 136.5 (C(9)).

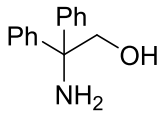
(*S*)-4-(1-amino-2-hydroxyethyl)phenol (46e)



An oven-dried three-neck flask was charged with a stirrer bar, $NaBH_4$ (0.756 g, 20.0 mmol) and anh. THF (20 mL) then cooled in an ice bath. To this stirring suspension was added a solution of iodine (2.54 g, 10.0 mmol) in anh. THF (10 mL) dropwise over 15 min [Caution hydrogen gas produced!]. After addition had finished and gas formation had ceased, 4-hydroxy-L-phenylglycine (1.67 g, 10.0 mmol) was added and the mixture heated to reflux for 14 h. The mixture was then cooled to rt and methanol added cautiously until a clear solution formed (~ 5 mL), stirred for 30 min and then evaporated. The resulting white paste was dissolved in aqueous potassium hydroxide (20%, 20 mL), stirred at rt for 4 h then neutralised to pH ~ 8 – 9. The product could not be extracted into an organic solvent and so the water was removed with a freeze drier. The resulting hygroscopic white solid was dissolved/suspended in ethanol:chloroform (1:1, 200 mL), stirred for 1 h then filtered and the solvent evaporated. The white solid was dissolved /suspended in ethanol:chloroform (1:9, 200

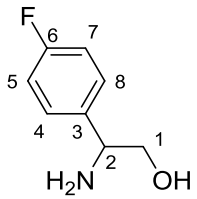
mL), stirred for 1 h then filtered. The filtrate was dried with $MgSO_4$ and evaporated to give **46e** as a hygroscopic white solid (1.5 g, 98%) which was used without any further purification.

2-amino-2,2-diphenylethanol (46g)[152]



To a suspension of 2-amino-2,2-diphenylacetic acid (1 g, 4.40 mmol) in anhydrous THF (10 mL) at rt was added borane-THF complex 1M in THF (11.00 mL, 11.00 mmol) dropwise over 5 min, then the suspension was stirred at 70 °C for 16.5 h. The solution was cooled to rt and then a further 10 mL of borane-THF complex 1M in THF was added and stirred at 70 °C for 6 h. After cooling to 20 ºC methanol (10 mL) was added cautiously then stirred for 30 min at rt. The solvent was then evaporated and the resulting white paste stirred with potassium hydroxide (20% aq w/w, 30 mL) for 16 h at 20 ºC, the solution was then extracted with ethyl acetate (3 × 25 mL), the combined organics were washed with brine, dried Na_2SO_4 and evaporated to give **46g** (0.863 g, 4.05 mmol, 92%) as a white solid, mp 118-120 °C; ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 2.35 (3H, br s, NH_2 and OH), 4.12 (2H, s, CH_2), 7.23 - 7.42 (10H, m, ArH); ^{13}C NMR (100 MHz, chloroform-*d*) δ ppm 62.4 (CNH_2), 70.2 (COH), 126.8 (*o*-Ph), 126.9 (*p*-Ph), 128.4 (*m*-Ph), 145.9 (*i*-Ph).

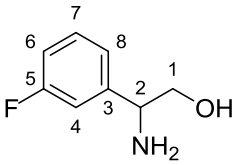
2-amino-2-(4-fluorophenyl)ethan-1-ol (46i)



A 3-neck flask was fitted with a magnetic stirrer and a reflux condenser and then flame dried. Under a nitrogen atmosphere $NaBH_4$ (0.224 g, 5.92 mmol, 2.0 eq) was added followed by anhydrous THF (10 ml). Then a dropping funnel was fitted and the mixture cooled to 0 °C in an ice bath. A solution of iodine (1.05 g, 2.96 mmol, 1.0 eq) in THF (10 ml) was added dropwise over 30 min, vigorous gas evolution was observed, after gas evolution had stopped 4-fluoro-DL-phenylglycine (0.5 g, 2.96 mmol, 1.0 eq) was added and the mixture was heated to reflux for 17 h. Then cooled to rt and methanol added slowly until the solution became clear. Stirred for 30 min and then the

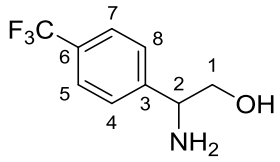
solvent was evaporated. The resulting white paste was dissolved in 30 ml of 20% aq. potassium hydroxide and stirred for 6 h then extracted with dichloromethane (3 × 30 ml). The combined organics were washed with brine (20 mL then 75 mL), dried Na_2SO_4 and then evaporated to give a white solid which was impure but used without further purification (0.22 g, 1.42 mmol, 48%); ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 2.51 (3H, br s, NH_2 and OH), 3.54 (1H, dd, *J*=10.8, 8.3 Hz, OC*H*H'), 3.72 (1H, dd, *J*=10.8, 4.3 Hz, OCH*H'*), 4.07 (1H, dd, *J*=8.3, 4.3 Hz, PhCH), 7.05 (2H, t, *J*=8.8 Hz, ArH), 7.32 (2H, dd, *J*=8.5, 5.5 Hz, ArH).

2-amino-2-(3-fluorophenyl)ethanol (46j)[153]



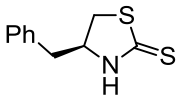
To a suspension of 2-amino-2-(3-fluorophenyl)acetic acid (1 g, 5.91 mmol) in anhydrous THF (10 mL) at rt was added borane-THF complex (1M in THF, 14.78 mL, 14.78 mmol) dropwise over 5 min, then the solution was stirred at 70 °C for 15.5 h. After cooling to 20 ºC, methanol (10 mL) was added cautiously, then stirred for 30 min. The solvent was then evaporated and the resulting white paste stirred with potassium hydroxide (20% aq w/w, 30 mL) for 4 h at 20 ºC, then extracted with ethyl acetate (2 × 30 mL). The combined organic layers were dried Na_2SO_4, and evaporated to give a yellow oil (0.9 g) which was purified by flash column chromatography on silica gel (2-10% (2M ammonia/methanol) in dichloromethane) to give a colourless oil (390 mg), which was purified again by MDAP to give **46j** (177 mg, 1.141 mmol, 19%) as a white solid, mp 86-88 °C; ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 2.17 (3H, br s, NH_2 and OH), 3.55 (1H, dd, *J*=10.9, 8.0 Hz, H(2)), 3.75 (1H, dd, *J*=10.9, 4.3 Hz, H(1)), 4.07 (1H, dd, *J*=8.0, 4.3 Hz, H(1)'), 6.88 - 7.02 (1H, m, ArH), 7.04 - 7.15 (2H, m, ArH), 7.26 - 7.36 (1H, m, ArH); ^{13}C NMR (100 MHz, chloroform-*d*) δ ppm 57.0 (s, C(2)), 67.8 (s, C(1)), 113.5 (d, *J*=21.6 Hz, C(4)), 114.3 (d, *J*=21.6 Hz, C(6)), 122.1 (d, *J*=3.2 Hz, C(8)), 130.1 (d, *J*=8.0 Hz, C(7)), 145.4 (d, *J*=6.4 Hz, C(3)), 163.0 (d, *J*=246.1 Hz, C(5)).

2-amino-2-(4-(trifluoromethyl)phenyl)ethanol (46k)[154]



To a suspension of 2-amino-2-(4-(trifluoromethyl)phenyl)acetic acid (1.5 g, 6.84 mmol) in anhydrous THF (15 mL) at rt was added borane-THF complex (1M in THF, 17.11 mL, 17.11 mmol) dropwise over 5 min, then the solution was stirred at 70 °C for 16.5 h. After cooling to 20 ºC methanol (10 mL) was added cautiously, then stirred for 30 min. The solvent was evaporated and the resulting yellow paste stirred with potassium hydroxide (20% aq w/w, 30 mL) for 4 h at 20 ºC, then extracted with ethyl acetate (3 × 30 mL), the combined organic layers were washed with brine, dried Na_2SO_4 and evaporated to give a yellow semi-solid (1.3 g). Purification by flash column chromatography on silica gel (2-10% (2M ammonia/methanol) in dichloromethane) gave **46k** (0.646 g, 3.15 mmol, 46%) as a white solid, mp 110-116 °C; ^{1}H NMR (500 MHz, chloroform-*d*) δ ppm 2.14 (3H, br s, NH_2 and OH), 3.58 (1H, dd, *J*=10.7, 8.0 Hz, H(2)), 3.78 (1H, dd, *J*=10.7, 4.1 Hz, H(1)), 4.16 (1H, dd, *J*=8.0, 4.1 Hz, H(1)'), 7.49 (2H, d, *J*=8.1 Hz, H(4 and 8)), 7.63 (2H, d, *J*=8.1 Hz, H(5 and 7)); ^{13}C NMR (125 MHz, chloroform-*d*) δ ppm 57.0 (s, C(2)), 67.8 (s, C(1)), 124.1 (q, *J*=271.9 Hz, CF_3), 125.5 (q, *J*=3.7 Hz, C(5 and 7)), 126.9 (s, C(4 and 8)), 129.8 (q, *J*=32.5 Hz, C(6)), 146.6 (s, C(3)); *m/z* (ESI$^+$) 206 ([M+H]$^+$, 100%); HRMS $C_9H_{11}NOF_3^+$ requires: 206.0787, found: 206.0790.

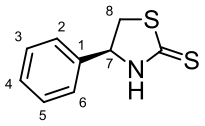
(4*S*)-4-benzyl-1,3-thiazolidine-2-thione (47a)[155]



To a mix of L-Phenylalinol **46a** (1.16 g, 7.67 mmol, 1.0 eq) in 1M aq. potassium hydroxide (38 ml) was added carbon disulfide (2.32 ml, 38.4 mmol, 5.0 eq). The resulting solution was stirred at reflux for 18 h then cooled to rt and extracted with dichloromethane (3 × 25 ml). The combined organic layers were dried $MgSO_4$ and evaporated to give **47a** as a white solid (1.5 g, 7.17 mmol, 93%), mp 72-74 °C (lit. 93-94 °C)[155]; ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 2.90 - 3.11 (2H, m, CH_2Ph), 3.31 (1H, dd, *J*=11.1, 6.7 Hz, C*H*H`S), 3.57 (1H, dd, *J*=11.1, 7.6 Hz, CH*H*`S), 4.38 - 4.55 (1H, m, CHN), 7.12 - 7.44 (5H, m, ArH), 7.81 (1H, br s, NH); ^{13}C NMR (75 MHz,

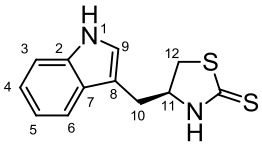
chloroform-*d*) δ ppm 38.2 (CH_2Ph), 40.1 (CH_2S), 65.1 (CHN), 127.5, 129.1, 129.2, 135.9 (4 ArC), 201.0 (C=S).

(4*S*)-4-phenyl-1,3-thiazolidine-2-thione (47b)[155]



To a mix of impure **(*S*)-46b** (0.80 g, 5.8 mmol, 1.0 eq) in 1M aq. potassium hydroxide (30 ml) was added carbon disulfide (1.75 ml, 29 mmol, 5.0 eq). The resulting solution was stirred at 80 °C (should have been at 100 °C) for 18 h then cooled to rt and extracted with dichloromethane (3 × 30 ml). The combined organic layers were washed with water (30 ml), dried $MgSO_4$ and evaporated. Purification by column chromatography on silica gel (hexane:ethyl acetate, 4:1) gave **47b** as a white solid (0.293 g, 1.50 mmol, 26%), mp 125-126 °C (lit. 125-127 °C)[156]; ν_{max} (cm^{-1}) 3125 (NH); ^{1}H NMR (500 MHz, chloroform-*d*) δ ppm 3.50 (1H, dd, *J*=11.3, 8.3 Hz, H(8)), 3.85 (1H, dd, *J*=11.3, 8.0 Hz, H(8)`), 5.32 (1H, t, *J*=8.2 Hz, H(7)), 7.32 - 7.49 (5H, m, ArH), 7.77 (1H, br s, NH); ^{13}C NMR (125 MHz, chloroform-*d*) δ ppm 41.6 (C(8)), 67.5 (C(7)), 126.3 (C(2 and 6)), 129.3 (C(4)), 129.4 (C(3 and 5)), 138.0 (C(4)), 201.7 (C=S).

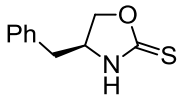
(4*S*)-4-(1*H*-indol-3-ylmethyl)-1,3-thiazolidine-2-thione (47c)



To a mix of impure **46c** (0.34 g, 1.79 mmol, 1.0 eq) in 1M aq. potassium hydroxide (9 ml) was added carbon disulfide (0.54 ml, 8.95 mmol, 5.0 eq). The resulting solution was stirred at reflux for 18 h then cooled to rt and extracted with dichloromethane (3 × 10 ml). The combined organic layers were washed with brine, dried $MgSO_4$ and evaporated. Purification by column chromatography on silica gel (hexane:ethyl acetate, 1:1) gave **47c** as a white solid (0.161 g, 0.65 mmol, 36%), mp 73-75 °C; ν_{max} (cm^{-1}) 3310 (NH), 3146 (NH); ^{1}H NMR (500 MHz, chloroform-*d*) δ ppm 3.12 (1H, dd, *J*=14.3, 6.1 Hz, H(10)), 3.16 (1H, dd, *J*=14.3, 7.7 Hz, H(10)`), 3.35 (1H, dd, *J*=11.1, 6.6 Hz, H(12)), 3.61 (1H, dd, *J*=11.1, 7.8 Hz, H(12) `), 4.51 - 4.60 (1H, m, H(11)), 7.08 (1H, d, *J*=2.4 Hz, (H(2)), 7.16 (1H, td, *J*=7.4, 0.9 Hz, H(6)), 7.24 (1H, td, *J*=7.7, 0.9 Hz, H(7)), 7.41 (1H, d, *J*=8.2 Hz, H(5)), 7.52 - 7.61 (2H, m, H(8) and CSNH), 8.22 (1H, br s, indole NH); ^{13}C NMR (125 MHz, chloroform-*d*) δ ppm 30.1

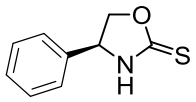
(C(10)), 38.5 (C(12)), 64.4 (C(11)), 110.3 (C(3)), 111.7 (C(8)), 118.4 (C(7)), 120.1 (C(5)), 122.7 (C(6)), 123.1 (C(2)), 126.9 (C(4)), 136.4 (C(9)), 200.9 (C=S); *m/z* (EI^+) 248 (M^+, 100%); HRMS $C_{12}H_{12}N_2S_2^+$ requires: 248.0436, found: 248.0439.

(4*S*)-4-benzyl-1,3-oxazolidine-2-thione (48a) [157]



To a mixture of L-phenylalinol **46a** (1.64 g, 10.9 mmol, 1 eq), potassium carbonate (0.753 g, 5.55 mmol, 0.5 eq) and CS_2 (1.32 ml, 21.8 mmol, 2 eq) in absolute ethanol (11 ml) stirred and heated to 50 °C was added 30% aq. hydrogen peroxide (1.85 ml, 16.4 mmol, 1.5 eq) dropwise [Caution: Exotherm!]. After completion of addition the mixture was cooled to rt and filtered. The filtrate was diluted with ethyl acetate (75 ml) washed with water (acidified with 2M hydrochloric acid as layers were not separating) (3 × 15 ml) and brine, then dried Na_2SO_4 and evaporated to give oxazolidine-2-thione **48a** as a colourless viscous oil (2.07 g, 10.7 mmol, 98%); ν_{max} (cm^{-1}) 3179 (NH), 1494 (C=S); ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 2.87 - 3.04 (2H, m, CH_2Ph), 4.28 - 4.38 (1H, m, CH), 4.42 (1H, dd, *J*=8.9, 6.4 Hz, C*H*H'O), 4.70 (1H, app. t, *J*=8.8 Hz, CH*H'*O), 7.20 (2H, d, *J*=7.0 Hz ArH), 7.26 - 7.40 (3H, m, ArH), 7.88 (1H, br s, NH); ^{13}C NMR (125 MHz, chloroform-*d*) δ ppm 40.6 (CH_2Ph), 57.9 (CH), 74.9 (CH_2O), 127.6, 129.1, 129.3, 135.3 (4 ArC), 189.7 (C=S).

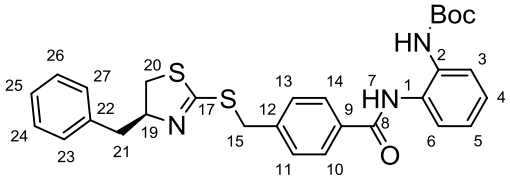
(*S*)-4-phenyloxazolidine-2-thione (48b)[157]



To a mixture of L-phenylglycinol **(*S*)-46b** (0.2 g, 1.46 mmol, 1 eq), potassium carbonate (0.101 g, 0.729 mmol, 0.5 eq) and CS_2 (0.176 ml, 2.92 mmol, 2 eq) in absolute ethanol (1.5 ml) stirred and heated to 50 °C was added 30% aq. hydrogen peroxide (0.248 ml, 2.19 mmol, 1.5 eq) dropwise [Caution: Exotherm!]. After completion of addition the mixture was cooled to rt and filtered. The filtrate was diluted with ethyl acetate (10 ml) washed with water (acidified with 2M hydrochloric acid as layers were not separating) (2 × 5 ml) and brine, then dried $MgSO_4$ and evaporated to give oxazolidine-2-thione **48b** as a white solid (0.21 g, 1.17 mmol, 80%), mp 117-118 °C (lit.[157] 120-121 °C); ν_{max} (cm^{-1}) 3172 (NH); ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 4.48 (1H, dd, *J*=9.0, 7.0 Hz, C*H*H'O), 5.00 (1H, t, *J*=9.0 Hz, CH*H'*O), 5.15 (1H,

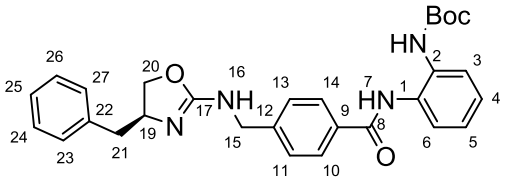
dd, *J*=9.0, 7.0 Hz, CHPh), 7.26 - 7.48 (5H, m, ArH), 8.15 (1H, br s, NH); ^{13}C NMR (125 MHz, chloroform-*d*) δ ppm 60.6 (CH_2O), 78.1 (CHPh), 126.6 (*m*-Ph), 129.6 (*p*-Ph), 129.8 (*o*-Ph), 138.3 (*i*-Ph), 190.3 (C=S).

tert-butyl _N_-(2-{[4-({[(4*S*)-4-benzyl-4,5-dihydro-1,3-thiazol-2-yl]sulfanyl}methyl)benzene]amido}phenyl)carbamate (49a)



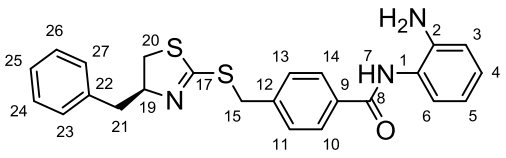
To a solution of the benzyl chloride **43** (0.92 g, 2.55 mmol, 1.1 eq) in acetone (15 ml) was added sodium iodide (0.765 g, 5.1 mmol, 2.2 eq) then stirred at reflux for 2 h (the round bottomed flask, oil bath and condenser were wrapped in foil to keep the benzyl iodide in the dark). After 2 h the mixture was cooled to rt and a solution of thiazolidine-2-thione **47a** (0.485 g, 2.32 mmol, 1.0 eq) in dichloromethane (10 ml) was added followed by potassium carbonate (0.481 g, 3.48 mmol, 1.5 eq). The resulting mixture was stirred at rt for 28 h (in the dark). The mixture was then evaporated then dissolved/suspended in ethyl acetate and filtered then evaporated. The residue was purified by column chromatography (dichloromethane to 5% EtOAC:dichloromethane) to give **49a** as a white solid (0.751 g, 1.4 mmol, 61%), mp 67-69 °C; ν_{max} (cm^{-1}) 3275 (NH), 1693 (C=O), 1656 (C=O); ^{1}H NMR (500 MHz, chloroform-*d*) δ ppm 1.50 (9H, s, $C(CH_3)_3$), 2.77 (1H, dd, *J*=13.6, 8.5 Hz, H(21)), 3.12 - 3.19 (2H, m, H(21)' and H(20)), 3.35 (1H, dd, *J*=10.7, 7.9 Hz, H(20)'), 4.36 (1H, d, *J*=13.6 Hz, H(15)), 4.43 (1H, d, *J*=13.6 Hz, H(15)'), 4.65 - 4.75 (1H, m, NCH, H(19)), 6.88 (1H, br s, BocNH), 7.10 - 7.28 (6H, m, ArH), 7.32 (2H, t, *J*=7.3 Hz, H(24 and 26)), 7.45 (2H, d, *J*=8.2 Hz, H(11 and 13)), 7.74 (1H, d, *J*=7.9 Hz, H(6)), 7.90 (2H, d, *J*=8.2 Hz, H(10 and 14)), 9.18 (1H, br s, H(7)); ^{13}C NMR (125 MHz, chloroform-*d*) δ ppm 28.4 ($C(CH_3)_3$), 36.6 (C(15)), 39.4 (C(20)), 40.2 (C(21)), 77.5 (C(19)), 81.5 ($OC(CH_3)_3$), 124.6 (C(3)), 125.9 (C(6)), 126.0 (C(4)), 126.1 (C(5)), 126.7 (C(24)), 127.7 (C(10 and 14)), 128.6 (C(11 and 13)), 129.4 (C(22 and 26)), 129.4 (C(23 and 25)), 130.1 (C(9)), 130.9 (C(2)), 133.4 (C(1)), 138.3 (C(21)), 141.2 (C(12)), 154.7 (CO_2N), 165.3 (C(17) and C(8)); *m/z* (EI^+) 533 (M^+, 35%); HRMS $C_{29}H_{31}N_3O_3S_2^+$ requires: 533.1801, found: 533.1804.

***tert*-butyl *N*-(2-{[4-({[(4*S*)-4-benzyl-4,5-dihydro-1,3-oxazol-2-yl]sulfanyl}methyl)benzene]amido}phenyl)carbamate (50a)**



To a solution of benzyl chloride **43** (0.512 g, 1.42 mmol, 1.1 eq) in acetone (10 ml) was added sodium iodide (0.427 g, 2.85 mmol, 2.2 eq) then stirred at reflux for 2 h (wrapped in foil to block out light). Cooled to rt and a solution of oxazolidine-2-thione **48a** (0.25 g, 1.29 mmol, 1 eq) in acetone (5 ml) was added and potassium carbonate (0.268 g, 1.94 mmol, 1.5 eq) was added then stirred at rt for 23 h. The solvent was evaporated then the residue was dissolved in ethyl acetate and filtered. The filtrate was evaporated and the resulting yellow oil was purified by column chromatography on silica gel (5 to 10% ethyl acetate/dichloromethane) to give benzamide **50a** as a white solid (0.434 g, 0.839 mmol, 59%), mp 59-61 °C; ν_{max} (cm^{-1}) 3280 (NH), 1696 (C=O), 1656 (C=O); ^{1}H NMR (500 MHz, chloroform-*d*) δ ppm 1.50 (9H, s, $C(CH_3)_3$), 2.67 (1H, td, *J*=13.8, 8.4 Hz, C*H*H'Ph), 3.09 (1H, td, *J*=13.8, 6.1 Hz, CH*H'*Ph), 4.08 (1H, app. t, *J*=8.0 Hz, C*H*H'O), 4.22 - 4.35 (3H, m, SCH_2 and CH*H'*O), 4.39 - 4.47 (1H, m, CH), 6.89 (1H, br s, ArCONH), 7.10 - 7.16 (1H, m, ArH), 7.17 - 7.27 (5H, m, ArH), 7.27 - 7.33 (2H, m, ArH), 7.48 (2H, d, *J*=8.0 Hz, ArH), 7.75 (1H, t, *J*=5.6 Hz, ArH), 7.91 (2H, d, *J*=8.0 Hz, ArH), 9.20 (1H, br s, OCONH); ^{13}C NMR (125 MHz, chloroform-*d*) δ ppm 28.4 ($C(CH_3)_3$), 35.9 (CH_2Ar), 41.7 (CH_2Ph), 67.8 (CH_2O), 73.7 (CH), 81.5 ($OC(CH_3)_3$), 124.6, 125.9, 126.1, 126.7, 127.8, 128.6, 129.3, 130.0, 130.9, 133.5, 137.7, 141.2 (12 ArC), 154.7 (ONC=O), 165.1 (C=N), 165.3 (ArNC=O) [2 aromatic carbons overlapping with other aromatic carbons]; *m/z* (ES^+) 518 ($[M+H]^+$, 33%), 462 ($[M+2H-C(CH_3)_3]^+$, 100%); HRMS $C_{29}H_{32}N_3O_4S^+$ requires: 518.2114, found: 518.2093.

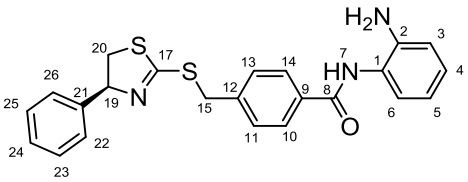
***N*-(2-aminophenyl)-4-(([(4*S*)-4-benzyl-4,5-dihydro-1,3-thiazol-2-yl]sulfanyl)methyl)benzamide (51a)**



To a solution of **49a** (0.30 g, 0.562 mmol) in dichloromethane (2.5 ml) was

added trifluoroacetic acid (0.5 ml) the solution was stirred at rt for 2.5 h, then evaporated and azeotroped with toluene four times. The residue was dissolved in dichloromethane (15 ml) and washed with sat. aq. sodium hydrogen carbonate (15 ml) then the aqueous layer was extracted with dichloromethane (10 ml), the combined organic layers were dried $MgSO_4$ and concentrated to give a cream solid that was purified by column chromatography on silica gel (hexane:ethyl acetate, 3:2) and then recrystallized from dichloromethane and hexane to give **51a** as a white solid (0.135 g, 0.311 mmol, 55%), mp 135-137 °C; $[\alpha]_D^{20}$ -20.6 (*c* 1.0, methanol); ν_{max} (cm^{-1}) 3274 (NH), 1635 (C=O), 1603 (C=N); ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 2.79 (1H, dd, *J*=13.6, 8.8 Hz, H(21)), 3.11 - 3.23 (2H, m, H(21)' and H(20)), 3.36 (1H, dd, *J*=10.9, 7.9 Hz, H(20)'), 3.84 (2H, br s, NH_2), 4.37 (1H, d, *J*=13.6 Hz, H(15)), 4.42 (1H, d, *J*=13.6 Hz, H(15)), 4.65 - 4.76 (1H, m, H(19)), 6.78 - 6.91 (2H, m, H(5) and H(3)), 7.09 (1H, t, *J*=7.4 Hz, H(4)), 7.19 - 7.39 (6H, m, ArH), 7.46 (2H, d, *J*=7.7 Hz, H(11 and 13)), 7.83 (2H, d, *J*=7.7 Hz, H(10 and 14)), 8.06 (1H, br s, H(7)); ^{13}C NMR (125 MHz, chloroform-*d*) δ ppm 36.5 ($ArCH_2S$), 39.5 (CH_2S), 40.2 (CH_2Ph), 77.8 (CH), 118.6, 120.0, 124.7, 125.4, 126.7, 127.4, 127.7, 128.6, 129.4, 129.5, 133.2, 138.5, 140.5, 141.5 (14 ArC), 163.9 C=N, 165.6 (C=O); *m/z* (ES^+) 434 ($[M+H]^+$, 100%); HRMS $C_{24}H_{24}N_3OS_2^+$ requires: 434.1361, found: 434.1360.

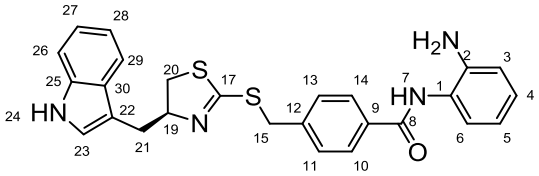
N-(2-aminophenyl)-4-({[(4*S*)-4-phenyl-4,5-dihydro-1,3-thiazol-2-yl]sulfanyl}methyl)benzamide (51b)



To a solution of **47b** (83 mg, 0.425 mmol, 1.0 eq) and benzyl chloride **54** (110 mg, 0.422 mmol, 1.0 eq) in acetone (4 ml) was added potassium carbonate (88 mg, 0.638 mmol, 1.5 eq) and then stirred at reflux for 17 h. Cooled to rt and evaporated and then partitioned between water (15 ml) and ethyl acetate (15 ml), the aq. layer was extracted with ethyl acetate (10 ml) then the combined organics were washed with brine (10 ml), dried Na_2SO_4, and evaporated to give a beige solid. Purification by column chromatography on silica gel (dichloromethane:ethyl acetate, 7:1) and then again (hexane:ethyl acetate, 3:2) gave **51b** as a white solid (114 mg, 0.262 mmol, 62%), mp 113-114 °C; $[\alpha]_D^{20}$ +75.5 (*c* 1.0, methanol); ν_{max} (cm^{-1}) 3276 (NH), 1647 (C=O), 1614 (C=N); ^{1}H NMR (500 MHz, chloroform-*d*) δ ppm 3.30 (1H, dd, *J*=10.9, 9.0 Hz,

C(20)*H*H`), 3.63 (2H, br s, $NH_2$), 3.78 (1H, dd, *J*=10.9, 8.3 Hz, C(20)H*H`*), 4.40 (1H, d, *J*=13.7 Hz, C(15)*H*H`), 4.46 (1H, d, *J*=13.7 Hz, C(15)H*H`*), 5.49 (1H, t, *J*=8.6 Hz, C(19)H), 6.74 - 6.85 (2H, m, H(5) and H(3)), 7.08 (1H, t, *J*=7.3 Hz, H(4)), 7.24 - 7.32 (4H, m, ArH), 7.32 - 7.38 (2H, m, ArH), 7.46 (2H, d, *J*=7.9 Hz, H(11 and 13)), 7.79 (2H, d, *J*=7.9 Hz, (H(10 and 14)), 8.01 (1H, br s, NH); ^{13}C NMR (125 MHz, chloroform-*d*) δ ppm 36.5 (C(20)), 43.1 (C(15)), 79.6 (C(19)), 118.5 (C(3)), 119.9 (C(5)), 124.6 (C(1)), 125.4 (C(6)), 126.6 (C(10 and 14)), 127.4 (C(4)), 127.7 (C(22 and 26)), 127.9 (C(24)), 128.7 (C(11 and 13)), 129.6 (C(23 and 25)), 133.3 (C(9)), 140.8 (C(2)), 141.5 (C(21)), 141.6 (C(12)), 165.2 (C(17)), 165.6 (C(8)); *m/z* (EI^+) 419 (M^+, 100%); HRMS $C_{23}H_{21}N_3OS_2^+$ requires: 419.1121, found: 419.1125.

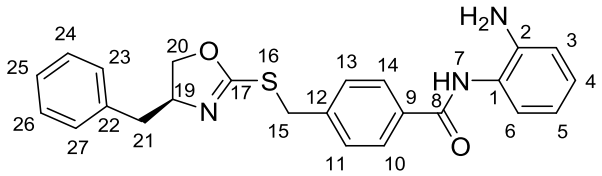
N-(2-aminophenyl)-4-({[(4*S*)-4-(1*H*-indol-3-ylmethyl)-4,5-dihydro-1,3-thiazol-2-yl]sulfanyl}methyl)benzamide (51c)



To a solution of **47c** (0.229 g, 0.920 mmol, 1.2 eq) and benzyl chloride **54** (0.2 g, 0.767 mmol, 1.0 eq) in acetone (4 ml) was added potassium carbonate (0.159 g, 1.15 mmol, 1.5 eq) and then stirred at reflux for 16.5 h. Cooled to rt and evaporated and then partitioned between water (25 ml) and ethyl acetate (25 ml), the aq. layer was extracted with ethyl acetate (10 ml) then the combined organics were washed with brine (10 ml), dried $MgSO_4$, and evaporated to give a beige solid. Purification by column chromatography on silica gel (hexane:ethyl acetate, 1:1) gave **51c** as a white solid (0.311 g, 0.658 mmol, 86%), mp 138-140 °C; $[\alpha]_D^{20}$ -16.5 (*c* 1.0, methanol); ν_{max} (cm^{-1}) 3379 (NH), 3324 (NH), 1638 (C=O); ^{1}H NMR (500 MHz, chloroform-*d*) δ ppm 2.96 (1H, dd, *J*=14.6, 8.3 Hz, C(21)*H*H`), 3.13 - 3.27 (2H, m, C(20)H*H`* and C(21)H*H`*), 3.34 (1H, dd, *J*=10.8, 8.0 Hz, C(20)*H*H`), 3.81 (2H, br s, NH_2), 4.30 (1H, d, *J*=13.6 Hz, C(15)*H*H`), 4.40 (1H, d, *J*=13.6 Hz, C(15)H*H`*), 4.82 (1H, app. quin, *J*=7.1 Hz, H(19)), 6.76 - 6.87 (2H, m, H(5) and H(3)), 6.91 (1H, s, H(23)), 7.03 - 7.15 (2H, m, H(4) and H(28)), 7.18 (1H, t, *J*=7.5 Hz, H(27)), 7.26 (1H, d, *J*=7.9 Hz, H(6)), 7.33 (1H, d, *J*=8.0 Hz, H(26)), 7.39 (2H, d, *J*=8.0 Hz, H(11 and 13)), 7.64 (1H, d, *J*=7.9 Hz, H(29)), 7.72 (2H, d, *J*=8.0 Hz, H(10 and 14)), 7.98 (1H, br s, NH), 8.36 (1H, br s, NH); ^{13}C NMR (125 MHz, chloroform-*d*) δ ppm 29.8 (C(21)), 36.4 (C(20)), 39.8 (C(15)), 77.1 (C(19)), 111.4 (C(26)), 112.4 (C(22)), 118.4 (C(3)), 118.9 (C(29)), 119.4 (C(27)), 119.8 (C(5)),

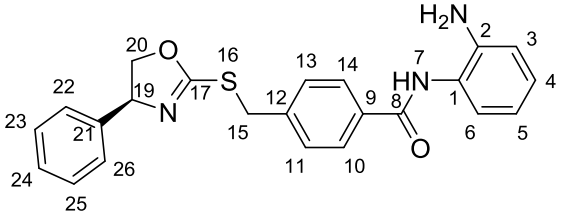
122.1 (C(28)), 122.9 (C(23)), 124.4 (C(1)), 125.6 (C(4)), 127.5 (C(6)), 127.6 (C(11 and 13)), 127.7 (C(30)), 129.5 (C(10 and 14)), 133.1 (C(9)), 136.3 (C(25)), 141.0 (C(2)), 141.6 (C(12)), 163.5 C((17)), 165.9 C((8)); *m/z* (EI^+) 472 (M^+, 10%), 208 ([M+H-(indoleCH_2)-($CONHC_6H_5NH_2$)]$^+$, 100%), 130 ([indole-CH_2]$^+$, 100%); HRMS $C_{26}H_{24}N_4OS_2^+$ requires: 472.1386, found: 472.1393.

(*S*)-*N*-(2-aminophenyl)-4-(((4-benzyl-4,5-dihydrooxazol-2-yl)thio)methyl)benzamide (52a)



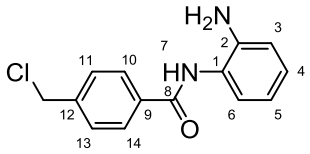
To a solution of thione **48a** (84 mg, 0.437 mmol, 1.2 eq) and benzyl chloride **54** (95 mg, 0.364 mmol, 1.0 eq) in acetone (4 mL) was added potassium carbonate (75 mg, 0.543 mmol, 1.5 eq), then mixture was then stirred at reflux for 16.5 h and evaporated. The residue was partitioned between water (15 mL) and ethyl acetate (20 mL) the layers were separated, and the aqueous layer was extracted with ethyl acetate (15 mL). The combined organic layers were washed with brine, dried $MgSO_4$ and evaporated to give an orange solid (175 mg) that was purified by column chromatography on silica gel (ethyl acetate:hexane, 1:1) to give **52a** as a cream solid (106 mg, 0.254 mmol, 70%), mp 137-140 °C; $[\alpha]_D^{25}$ -32.0 (*c* 0.5, methanol); ν_{max} (cm^{-1}) 3274 (NH), 1649 (C=O); ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 2.66 (1H, dd, *J*=13.8, 8.3 Hz, H(21)), 3.08 (1H, dd, *J*=13.8, 5.5 Hz, H(21)'), 3.86 (2H, br s, NH_2), 4.03 - 4.12 (1H, m, H(20)), 4.19 - 4.35 (3H, m, H(20)', H(15) and H(15)'), 4.36 - 4.50 (1H, m, H(19)), 6.75 - 6.89 (2H, m, H(5) and H(3)), 7.02 - 7.13 (1H, m, H(4)), 7.14 - 7.35 (6H, m,), 7.48 (2H, d, *J*=8.0 Hz, H(11 and 13)), 7.83 (2H, d, *J*=8.0 Hz, H(10 and 14)), 7.96 (1H, br s, H(7)); ^{13}C NMR (125 MHz, chloroform-*d*) δ ppm 36.2 (C(21)), 42.0 (C(15)), 68.2 (C(20)), 74.0 (C(19)), 118.8 (C(3)), 120.2 (C(6)), 125.0 (C(1)), 125.6 (C(4)), 127.0 (C(5)), 127.7 (C(24)), 128.0 (C(11 and 13)), 129.0 (C(10 and 14)), 129.6 (C(22 and 26)), 129.8 (C(23 ad 25)), 133.7 (C(9)), 138.0 (C(2)), 141.1 (C(21)), 141.7 (C(12)), 165.3 (C(17)), 165.8 (C(8)); *m/z* (ESI^-) 416 ([M-H]$^-$, 100%); HRMS $C_{24}H_{22}N_3O_2S^-$ requires: 416.1438, found:416.1433.

(*S*)-*N*-(2-aminophenyl)-4-(((4-phenyl-4,5-dihydrooxazol-2-yl)thio)methyl)benzamide (52b)



To a solution of thione **48b** (185 mg, 1.03 mmol, 1.0 eq) and benzyl chloride **54** (269 mg, 1.03 mmol, 1.0 eq) in acetone (10 mL) was added potassium carbonate (213 mg, 1.56 mmol, 1.5 eq), then mixture was then stirred at reflux for 18 h and evaporated. The residue was partitioned between water (40 mL) and ethyl acetate (40 mL) the layers were separated, and the organic layer was washed with brine, dried $MgSO_4$ and evaporated to give an orange solid (420 mg) that was purified by column chromatography on silica gel (ethyl acetate:hexane, 1:1), and then purified again by column chromatography on silica gel (dichloromethane:ethyl acetate, 4:1 to 3:1) to give **52b** as a cream solid (66 mg, 0.163 mmol, 16%), mp 64-65 °C; $[\alpha]_D^{25}$ -14.0 (*c* 0.5, methanol); ν_{max} (cm^{-1}) 3292 (NH), 1653 (C=O); ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 3.91 (2H, br s, NH_2), 4.22 (1H, t, *J*=8.0 Hz, H(20), 4.31 - 4.46 (2H, m, H(15) and H(15)'), 4.75 (1H, t, *J*=8.9 Hz, H(20)'), 5.25 (6H, dd, *J*=9.5, 8.0 Hz, H(19)), 6.83 - 6.93 (2H, m, H(5) and H(3)), 7.13 (1H, t, *J*=7.3 Hz, H(4)), 7.21 (2H, d, *J*=7.0 Hz, ArH), 7.26 - 7.41 (6H, m, ArH), 7.56 (2H, d, *J*=8.0 Hz, ArH), 7.87 (3H, d, *J*=7.5 Hz, H(10 and 14) and H(7)); ^{13}C NMR (125 MHz, chloroform-*d*) δ ppm 35.9 (C(15)), 69.9 (C(19)), 76.6 (C(20)), 118.5 (C(3)), 119.8 (C(5)), 124.6 (C(1)), 125.4 (C(4)), 126.6 (C(11 and 13)), 127.4 (C(6)), 127.7 (C(22 and 26)), 127.9 (C(24)), 128.8 (C(23 and 25)), 129.5 (C(10 and 14)), 133.4 (C(9)), 140.8 (C(21)), 141.4 (C(21)), 141.9 (C(12)), 165.6 (C(17)), 166.0 (C(8)); *m/z* (CI) 404 ($[M+H]^+$, 100%); HRMS $C_{23}H_{22}N_3O_2S^+$ requires: 404.1427, found: 404.1426.

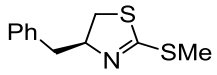
***N*-(2-aminophenyl)-4-(chloromethyl)benzamide (54)**



To a solution of Boc-protected benzylchloride **43** (0.361 g, 1.0 mmol) in dichloromethane (2.5 ml) was added trifluoroacetic acid (0.5 ml) and the resulting

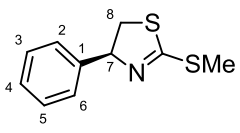
solution stirred at rt for 2.5 h. The solution was azeotroped with toluene two times, and the resulting yellow solid triturated with diethyl ether three times to give a white solid. Partitioned between ethyl acetate (30 ml) and sat. aq. sodium hydrogen carbonate (20 ml), organic layer washed with brine, dried Na_2SO_4 and evaporated to give **54** as a white solid (0.190 g, 0.727 mmol, 73%), mp 132-133 °C; ν_{max} (cm^{-1}) 3370 (NH), 3279 (NH), 1645 (C=O); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 4.83 (2H, s, CH_2), 4.90 (2H, br s, NH_2), 6.59 (1H, t, *J*=7.4 Hz, H(5)), 6.77 (1H, d, *J*=7.3 Hz, H(3)), 6.97 (1H, t, *J*=7.6 Hz, H(4)), 7.16 (1H, d, *J*=7.6 Hz, H(6)), 7.56 (2H, d, *J*=7.9 Hz, H(11 and 13)), 7.97 (2H, d, *J*=7.9 Hz, H(10 and 14)), 9.67 (1H, s, H(7)); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 45.5 (CH_2), 116.1 (C(3)), 116.2 (C(5)), 123.2 (C(6)), 126.6 (C(1)), 126.7 (C(4)), 128.1 (C(11 and 13)), 128.7 (C(10 and 14)), 134.5 (C(9)), 140.8 (C(12)), 143.1 (C(2)), 164.9 (C(8)); *m/z* (EI^+) 260 (M^+, 11%), 153 ([M-$PhNH_2NH$]$^+$, 30%); HRMS $C_{14}H_{13}N_2OCl^+$ requires: 260.0711, found: 260.0708.

(4*S*)-4-benzyl-2-(methylsulfanyl)-4,5-dihydro-1,3-thiazole (56a)



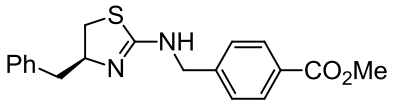
To a solution of **47a** (1.00 g, 4.78 mmol, 1.0 eq) in acetone (10 ml) was added iodomethane (0.36 ml, 5.73 mmol, 1.2 eq) and then stirred at reflux for 3 h. TLC indicated that some starting material remained so iodomethane (0.1 ml, 1.6 mmol, 0.3 eq) and stirred at reflux for a further 2 h. Then evaporated and partitioned between sat. aq. sodium hydrogen carbonate and dichloromethane, the organic layer was dried $MgSO_4$ and evaporated to give **56a** as a colourless oil (1.06 g, 4.73 mmol, 99%); ν_{max} (cm^{-1}) 1561 (C=N); ^{1}H NMR (500 MHz, chloroform-*d*) δ ppm 2.56 (3H, s, SCH_3), 2.74 (1H, dd, *J*=13.6, 9.1 Hz, C*H*H`Ph), 3.14 (1H, dd, *J*=10.8, 6.6 Hz, C*H*H`S), 3.19 (1H, dd, *J*=13.6, 5.0 Hz, CH*H*`Ph), 3.32 (1H, dd, *J*=10.8, 7.9 Hz, CH*H*`S), 4.64 - 4.73 (1H, m, CHN), 7.21 - 7.27 (3H, m, ArH), 7.29 - 7.34 (2H, m, ArH); ^{13}C NMR (125 MHz, chloroform-*d*) δ ppm 15.5 (CH_3), 39.3 (CH_2Ph), 40.2 (CH_2S), 77.9 (CHN), 126.6, 128.6, 129.4, 138.5 (4 ArC), 165.7 (C=N); *m/z* (CI^+) 224 ([M+H]$^+$, 100%); HRMS $C_{11}H_{14}NS_2^+$ requires 224.0568, found: 224.0569.

(4*S*)-2-(methylsulfanyl)-4-phenyl-4,5-dihydro-1,3-thiazole (56b)



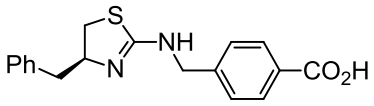
To a suspension of **47b** (0.20 g, 1.02 mmol, 1.0 eq) in acetone (2 ml) was added iodomethane (0.097 ml, 1.54 mmol, 1.5 eq). The mixture was stirred at reflux for 4 h under a nitrogen balloon then evaporated and the resulting white solid partitioned between sodium hydrogen carbonate (sat. aq., 15 ml) and dichloromethane (15 ml). The aqueous layer was extracted with dichloromethane (10 ml) and the combined organics were washed with brine (10 ml) dried $MgSO_4$ and concentrated to give **56b** as an orange oil (0.188 g, 0.898 mmol, 88%); ν_{max} (cm^{-1}) 1561 (C=N); ^{1}H NMR (500 MHz, chloroform-*d*) δ ppm 2.61 (3H, s, SCH_3), 3.30 (1H, dd, *J*=10.9, 9.0 Hz, H(8)H), 3.79 (1H, dd, *J*=10.9, 8.2 Hz, H(8)`), 5.49 (1H, app. t, *J*=8.6 Hz, H(7)), 7.23 - 7.40 (5H, m, ArH); ^{13}C NMR (125 MHz, chloroform-*d*) δ ppm 15.6 (SCH_3), 43.1 (C(8)), 79.7 (C(7)), 126.6 (C(3 and 5)), 127.8 (C(4)), 128.7 (C(2 and 6), 141.7 (C(1)), 167.0 (C=N); *m/z* (EI^+) 209 (M^+, 34%); HRMS $C_{10}H_{11}NS_2^+$ requires: 209.0327, found: 209.0333.

methyl 4-(([(4*S*)-4-benzyl-4,5-dihydro-1,3-thiazol-2-yl]amino)methyl)benzoate (57a)



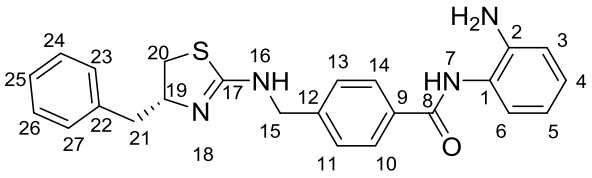
To a solution of dihydro-1,3-thiazole **56a** (0.24 g, 1.07 mmol, 1 eq) in ethanol (2.5 ml) was added methyl 4-(aminomethyl)benzoate hydrochloride (0.22 g, 1.07 mmol, 1 eq) and *N*,*N*-diisopropylethylamine (186 µl, 1.07 mmol, 1 eq). The resulting mixture was stirred at reflux for 24 h. Then concentrated and partitioned between ethyl acetate (25 ml) and sat. aq. sodium hydrogen carbonate (25 ml), the organic layer was washed with sat. aq. sodium hydrogen carbonate, dried with Na_2SO_4, and concentrated to give an orange oil that was purified by column chromatography on silica gel (4% methanol/dichloromethane) to give **57a** as a colourless oil (0.23 g, 0.676 mmol, 63%); ν_{max} (cm^{-1}) 1716 (C=O), 1610 (C=N); ^{1}H NMR (500 MHz, chloroform-*d*) δ ppm 2.74 (1H, dd, *J*=13.4, 8.7 Hz, CH*H'*Ph), 3.02 - 3.11 (2H, m, CH*H'*S and C*H*H'Ph), 3.26 (1H, dd, *J*=10.7, 6.9 Hz, C*H*H'S), 3.91 (3H, s, CO_2CH_3), 4.39 - 4.46 (2H, C*H*N and N*H*), 4.49 (1H, d, *J*=15.4 Hz, ArC*H*H'N), 4.54 (1H, d, *J*=15.4 Hz, ArCH*H'*N), 7.17 - 7.24 (3H, m, 3 ArH), 7.25 - 7.32 (2H, m, 2 ArH), 7.38 (2H, d, *J*=8.2 Hz, Ar-3,5 H), 8.00 (2H, d, *J*=8.2 Hz, Ar-2,6 H); ^{13}C NMR (125 MHz, chloroform-*d*) δ ppm 38.3 (CH_2S), 41.2 (CHPh), 49.6 (CO_2CH_3), 52.2 (CH_2N), 71.9 (CH), 126.5, 127.4, 128.6, 129.3, 129.3, 130.0, 138.6, 144.0 (8 ArC), 161.4 (C=N), 167.0 (C=O); *m/z* (ES^+) 341 ($[M+H]^+$, 100%); HRMS $C_{19}H_{21}N_2O_2S^+$ requires: 341.1324, found: 341.1324.

4-(([(4*S*)-4-benzyl-4,5-dihydro-1,3-thiazol-2-yl]amino)methyl)benzoic acid (58a)



To a solution of methyl ester **57a** (0.210, 0.617 mmol, 1 eq) in THF (3.5 ml) was added a solution of lithium hydroxide monohydrate (0.065 g, 1.54 mmol, 2.5 eq) in water (1.5 ml), then stirred at rt for 26 h. The THF was evaporated then diluted with water (25 ml) and extracted with ethyl acetate (25 ml) and 5% methanol in dichloromethane (2 × 25 ml). The combined organics were washed with brine, dried Na_2SO_4 and evaporated. The crude product was purified by column chromatography on silica gel (10% methanol/dichloromethane) to give the benzoic acid **58a** as a white solid (0.122 g, 0.375 mmol, 61%), mp 70-72 °C; ν_{max} (cm^{-1}) 1706 (C=O), 1633 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.91 (1H, dd, *J*=13.6, 7.8 Hz, C*H*H'Ph), 3.03 (1H, dd, *J*=13.6, 4.8 Hz, CH*H'*Ph), 3.25 (1H, dd, *J*=11.3, 5.8 Hz, C*H*H'S), 3.37 (1H, br s, NH), 3.52 (1H, dd, *J*=11.3, 7.8 Hz, (CH*H'*S), 4.50 - 4.61 (1H, m, CH), 4.65 - 4.80 (2H, m, NCH_2Ar), 7.15 - 7.34 (5H, m, ArH), 7.48 (2H, d, *J*=8.0 Hz, ArH), 7.97 (2H, d, *J*=8.0 Hz, ArH), 10.60 (1H, br s, CO_2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 34.6 (CH_2S), 38.7 (CH_2Ph), 47.9 (NCH_2Ar), 63.3 (CH), 126.7, 127.7, 128.4, 129.5, 129.6, 130.2, 136.3, 141.3 (8 ArC), 167.0 (C=O); *m/z* (ES^+) 327 ($[M+H]^+$, 100%); HRMS $C_{18}H_{19}N_2O_2S^+$ requires: 327.1167, found: 327.1170.

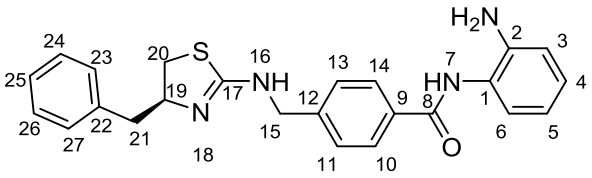
(*R*)-*N*-(2-aminophenyl)-4-(((4-benzyl-4,5-dihydrothiazol-2-yl)amino)methyl)benzamide ((*R*)-59a)



The thiourea **(*R*)-79a** (0.355 g, 0.664 mmol) was reacted according to general procedure B for 15 min. Purification of the residue by column chromatography on silica gel (ethyl acetate:hexane, 3:2 to 2:1) gave a cream solid (117 mg) which was recrystallized from chloroform to give **(*R*)-59a** as a white solid (62 mg, 22%), mp 143−144 °C; $[\alpha]_D^{20}$ +9.7 (c 1.0, methanol); ν_{max} (cm^{-1}) 3164 (NH), 1662 (C=O), 1615 (C=N); ^{1}H NMR (400 MHz, chloroform-*d*) δ 2.75 (1H, dd, J = 13.3, 8.8 Hz, H(20)), 2.98−3.17 (2H, m, H(20)' and H(21)), 3.28 (1H, dd, J = 10.7, 7.2 Hz, H(21)'), 3.90 (2H, br s, NH_2), 4.38−4.47 (1H, m, H(19)), 4.47−4.59 (2H, m, H(15) and H(15)'), 5.16 (1H,

br s, H(16)), 6.76−6.93 (2H, m, H(5) and H(3)), 7.11 (1H, td, J = 7.7, 1.0 Hz, H(4)), 7.17−7.35 (6H, m, H(24 and 27), H(6), H(24 and 26) and H(25)), 7.40 (2H, d, J = 8.0 Hz, H(11 and 13)), 7.85 (2H, d, J = 8.0 Hz, H(10 and 14)), 8.05 (1H, br s, H(7)); ^{13}C NMR (150 MHz, chloroform-*d*) δ ppm 38.5 (C(20)), 41.3 (C(21)), 49.6 (C(15)), 72.1 (C(19)), 118.5 (C(3)), 119.8 (C(5)), 124.7 (C(1)), 125.3 (C(4)), 126.5 (C(25)), 127.3 (C(6)), 127.7 (C(24 and 26)), 127.8 (C(11 and 13)), 128.6 (C(10 and 14)), 129.4 (C(23 and 27)), 133.2 (C(9)), 138.8 (C(2)), 140.8 (C(22)), 143.3 (C(12)), 161.0 (C(17)), 165.7 (C(8)); m/z (ES, %) 417 ([M+H]$^+$, 100); HRMS (CI) calcd for $C_{24}H_{25}N_4OS$ [M+H]$^+$ 417.1744, found 417.1723.

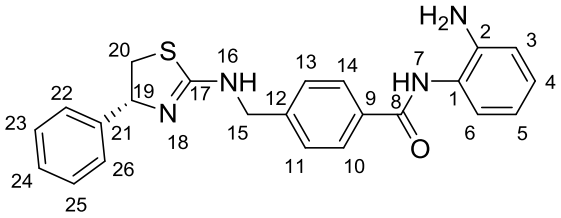
(*S*)-*N*-(2-aminophenyl)-4-(((4-benzyl-4,5-dihydrothiazol-2-yl)amino)methyl)benzamide ((*S*)-59a)



A solution of **(*S*)-79a** (0.135 g, 0.252 mmol) in conc. hydrochloric acid (5 ml) was heated at 90 °C under a reflux condenser and stirred for 15 min. Then sodium hydroxide (5M, aq) was added to pH ~ 9, and the product extracted with ethyl acetate (3 × 30 ml). The combined organics were washed with brine, dried Na_2SO_4, and evaporated. Purification by column chromatography on silica gel (1% methanol/ethyl acetate) gave a cream solid (68 mg) which was further purified by recrystallization from chloroform to give **(*S*)-59a** as a white solid (10 mg, 0.024 mmol, 10%), mp 144-145 °C; $[\alpha]_D^{20}$ -9.2 (*c* 1.0, methanol); ν_{max} (cm^{-1}) 3162 (NH), 1636 (C=O), 1614 (C=N); ^{1}H NMR (500 MHz, chloroform-*d*) δ ppm 2.72 (1H, dd, *J*=13.4, 8.8 Hz, H(20)), 3.00 - 3.10 (2H, m, H(20)` and H(21)), 3.24 (1H, dd, *J*=10.6, 7.2 Hz, H(21)`), 3.94 (3H, br s, NH_2 and H(16)), 4.34 - 4.42 (1H, m, H(19)), 4.47 (2H, m, H(15) and H(15)'), 6.74 - 6.86 (2H, m, H(5) and H(3)), 7.07 (1H, app. t, *J*=7.5 Hz, H(4)), 7.19 (2H, d, *J*=7.4 Hz, H(24 and 27)), 7.22 (1H, d, *J*=7.1 Hz, H(6)), 7.25 - 7.31 (3H, m, H(24 and 26) and H(25)), 7.34 (2H, d, *J*=7.7 Hz, H(11 and 13)), 7.80 (2H, d, *J*=7.7 Hz, H(10 and 14)), 8.13 (1H, br s, H(7)); ^{13}C NMR (150 MHz, chloroform-*d*) δ ppm 38.3 (C(20)), 41.3 (C(21)), 49.6 (C(15)), 71.9 (C(19)), 118.5 (C(3)), 119.8 (C(5)), 124.6 (C(1)), 125.5 (C(4)), 126.6 (C(25)), 127.3 (C(6)), 127.7 (C(24 and 26)), 127.8 (C(11 and 13)), 128.6 (C(10 and 14)), 129.4 (C(23 and 27)), 133.2 (C(9)), 138.8 (C(2)), 140.9 (C(22)), 143.2 (C(12)), 161.5 (C(17)), 165.8 (C(8)); *m/z* (ES$^+$) 417 ([M+H]$^+$, 100%); HRMS $C_{24}H_{25}N_4OS^+$ requires: 417.1749,

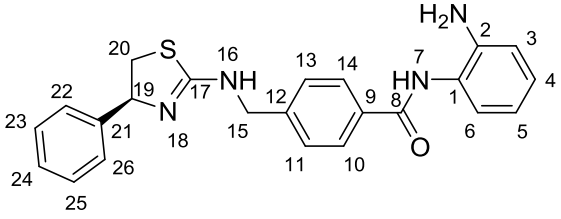
found: 417.1738.

(*R*)-*N*-(2-aminophenyl)-4-(((4-phenyl-4,5-dihydrothiazol-2-yl)amino)methyl)benzamide ((*R*)-59b)



The thiourea **(*R*)-79b** (0.345 g, 0.663 mmol) was reacted according to general procedure B for 45 min. Purification of the residue by column chromatography on silica gel (ethyl acetate:hexane, 8:1) afforded a cream solid (149 mg) which was recrystallized from chloroform to give **(*R*)-59b** as a white solid (62 mg, 23%), mp 155−156 °C; $[\alpha]_D^{20}$ −62.0 (c 1.0, methanol); ν_{max} (cm^{-1}) 3281 (NH), 1612 (C=O); ^{1}H NMR (400 MHz, chloroform-*d*) δ 3.23 (1H, dd, J = 10.5, 8.3 Hz, H(20)), 3.70 (1H, dd, J = 10.5, 7.5 Hz, H(20)'), 3.90 (2H, br s, NH_2), 4.47−4.60 (2H, m, H(15) and H(15)`), 5.28 (1H, t, J = 7.9 Hz, H(19)), 5.71 (1H, br s, H(16)), 6.78−6.91 (2H, m, H(5) and H(3)), 7.11 (1H, td, J = 7.6, 1.0 Hz, H(4)), 7.20−7.36 (6H, m, H(22 and 26), H(23 and 25), H(24) and H(6)), 7.40 (2H, d, J = 8.0 Hz, H(11 and 13)), 7.85 (2H, d, J = 7.8 Hz, H(10 and 14)), 8.05 (1H, br s, H(7)); ^{13}C NMR (150 MHz, chloroform-*d*) δ ppm 42.5 (C(20)), 49.3 (C(15)), 74.5 (C(19)), 118.5 (C(3)), 119.9 (C(6)), 124.7 (C(1)), 125.4 (C(4)), 126.5 (C(22 and 26)), 127.3 (C(24)), 127.6 (C(5)), 127.7 (C(23 and 25)), 127.8 (C(11 and 13)), 128.6 (C(10 and 14)), 133.2 (C(9)), 140.8 (C(2)), 143.1 (C(21)), 143.3 (C(12)), 162.2 (C(17)), 165.7 (C(8)); m/z (ES, %) 403 ($[M+H]^+$, 100); HRMS (CI) calcd for $C_{23}H_{23}N_4O_S$ $[M+H]^+$ 403.1587, found 403.1570.

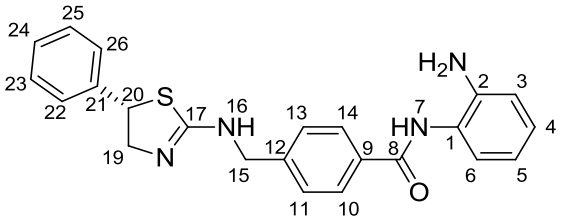
(*S*)-*N*-(2-aminophenyl)-4-(((4-phenyl-4,5-dihydrothiazol-2-yl)amino)methyl)benzamide ((*S*)-59b)



A solution of **(*S*)-79b** (0.460 g, 0.883 mmol) in conc. hydrochloric acid (15 ml) was heated at 90 °C under a reflux condenser and stirred for 75 min. Then sodium

hydroxide (5M, aq) was added to pH ~ 9, and the product extracted with ethyl acetate (100 mL + 2 × 50 ml). The combined organics were washed with brine, dried Na_2SO_4, and evaporated. Purification by column chromatography on silica gel (ethyl acetate:hexane, 7:1 to 8:1) gave a cream solid (257 mg) which was further purified by recrystallization from chloroform to give **(*S*)-59b** as a white solid (85 mg, 0.211 mmol, 24%), mp 150-151 °C; $[\alpha]_D^{20}$ +67.7 (*c* 1.0, methanol); ν_{max} (cm^{-1}) 3190 (NH), 1622 (C=O); ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 3.23 (1H, dd, *J*=10.6, 8.3 Hz, H(20)), 3.70 (1H, dd, *J*=10.6, 7.7 Hz, H(20)`), 3.89 (2H, br s, $NH_2$), 4.54 (2H, s, H(15) and H(15)`), 5.28 (1H, app. t, *J*=8.0 Hz, H(19)), 5.66 (1H, br s, H(16)), 6.79 - 6.90 (2H, m, H(5) and H(3)), 7.11 (1H, td, *J*=7.7, 1.5 Hz, H(4)), 7.22 - 7.36 (6H, m, H(22 and 26), H(23 and 25), H(24) and H(6)), 7.40 (2H, d, *J*=8.0 Hz, H(11 and 13)), 7.84 (2H, d, *J*=8.0 Hz, H(10 and 14)), 8.04 (1H, br s, H(7)); ^{13}C NMR (150 MHz, chloroform-*d*) δ ppm 42.5 (C(20)), 49.4 (C(15)), 74.4 (C(19)), 118.5 (C(3)), 119.9 (C(6)), 124.7 (C(1)), 125.4 (C(4)), 126.5 (C(22 and 26)), 127.3 (C(24)), 127.6 (C(5)), 127.7 (C(23 and 25)), 127.8 (C(11 and 13)), 128.6 (C(10 and 14)), 133.2 (C(9)), 140.9 (C(2)), 143.1 (C(21)), 143.3 (C(12)), 162.3 (C(17)), 165.8 (C(8)); *m/z* (CI^+) 403 ($[M+H]^+$, 21%), 385 ($[M+H\text{-water}]^+$, 100%); HRMS $C_{23}H_{22}N_4OS^+$ requires: 403.1593, found: 403.1585.

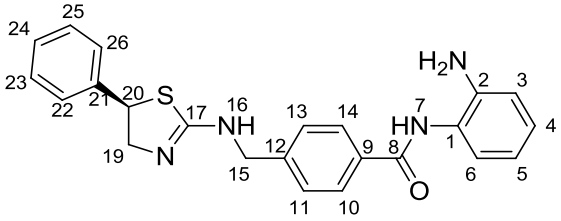
(*S*)-*N*-(2-aminophenyl)-4-(((5-phenyl-4,5-dihydrothiazol-2-yl)amino)methyl)benzamide ((*S*)-59d)



Thiourea **(*R*)-79d** (0.50 g, 0.96 mmol) was reacted according to general procedure B for 30 min. The residue was purified by column chromatography on silica gel (7% methanol/dichloromethane) and then recrystallized from ethanol and water twice to give **(*S*)-59d** as a white solid (0.190 g, 49%), mp 159-164 °C; $[\alpha]_D^{20}$ -35.3 (*c* 1.0, MeOH); ν_{max} (cm^{-1}) 3328 (NH), 1633 (C=O), 1620 (C=N); ^{1}H NMR (600 MHz, methanol-d_4) δ ppm 3.98 (1H, dd, *J*=13.2, 5.3 Hz, H(19)), 4.25 (1H, dd, *J*=13.2, 7.7 Hz, H(19)'), 4.55 (2H, s, H(15) and H(15)'), 4.99 - 5.06 (1H, m, H(20)), 6.78 (1H, t, *J*=7.5 Hz, H(5)), 6.91 (1H, d, *J*=7.9 Hz, H(3)), 7.08 (1H, t, *J*=7.5 Hz, H(4)), 7.19 (1H, d, *J*=7.5 Hz, H(6)), 7.22 - 7.27 (1H, m, H(24)), 7.28 - 7.40 (4H, m, H(23 and 25) and H(22 and 26)), 7.48 (2H, d, *J*=7.9 Hz, H(11 and 13)), 7.97 (2H, d, *J*=7.9 Hz, H(10 and 14)); ^{13}C

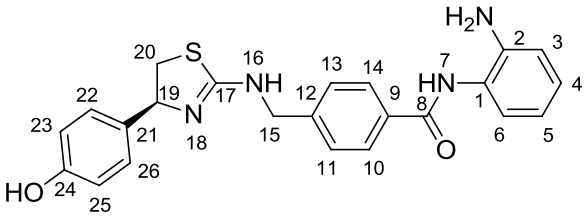
NMR (150 MHz, methanol-d_4) δ ppm 48.6 (C(15)), 56.3 (C(19)), 68.0 (C(20)), 118.9 (C(3)), 119.9 (C(6)), 125.5 (C(1)), 127.8 (C(4)), 128.3 (C(11 and 13)), 128.6 (C(10 and 14)), 128.7 (C(5)), 128.9 (C(24)), 129.2 (C(22 and 26)), 129.9 (C(23 and 25)), 134.5 (C(9)), 143.0 (C(2)), 143.9 (C(21)), 144.6 (C(12)), 164.9 (C(17)), 168.8 (C(8)); *m/z* (ES, %) 401 ([M-H]$^-$, 100); HRMS calcd. for $C_{23}H_{21}N_4OS$ [M-H]$^-$ 401.1436, found: 401.1446.

(*R*)-*N*-(2-aminophenyl)-4-(((5-phenyl-4,5-dihydrothiazol-2-yl)amino)methyl)benzamide ((*R*)-59d)



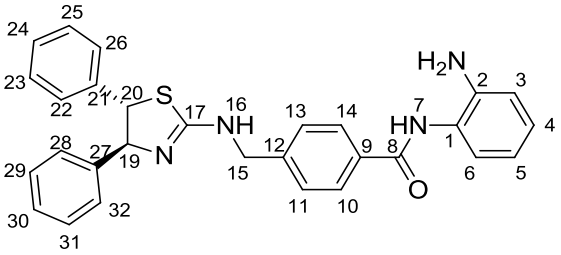
Thiourea **(*S*)-79d** (0.25 g, 0.480 mmol) was reacted according to general procedure B for 45 min. The residue was purified by column chromatography on silica gel (5% methanol/dichloromethane) and then recrystallized from ethanol and water to give **(*R*)-59d** as a white solid (41 mg, 21%), mp 161-163 °C; $[\alpha]_D^{20}$ +38.4 (*c* 1.0, MeOH); ν_{max} (cm^{-1}) 3250 (NH), 1610 (C=O); ^{1}H NMR (400 MHz, methanol-d_4) δ ppm 4.00 (1H, dd, *J*=13.2, 5.3 Hz, H(19)), 4.27 (1H, dd, *J*=13.2, 7.6 Hz, H(19)'), 4.57 (2H, s, H(15) and H(15)'), 5.04 (1H, dd, *J*=7.6, 5.3 Hz, H(20)), 6.80 (1H, td, *J*=7.7, 1.3 Hz, H(5)), 6.93 (1H, dd, *J*=8.0, 1.3 Hz, H(3)), 7.07 - 7.14 (1H, m, H(4)), 7.21 (1H, dd, *J*=7.9, 1.1 Hz, H(6)), 7.24 - 7.30 (1H, m, H(24)), 7.30 - 7.36 (2H, m, H(23 and 25)), 7.37 - 7.42 (2H, m, H(22 and 26)), 7.51 (2H, d, *J*=8.2 Hz, H(11 and 13)), 7.99 (2H, d, *J*=8.2 Hz, H(10 and 14)); ^{13}C NMR (150 MHz, methanol-d_4) δ ppm 48.5 (C(15)), 56.3 (C(19)), 68.3 (C(20)), 118.8 (C(3)), 119.7 (C(6)), 125.4 (C(1)), 127.7 (C(4)), 128.1 (C(11 and 13)), 128.4 (C(10 and 14)), 128.5 (C(5)), 128.7 (C(24)), 129.0 (C(22 and 26)), 129.7 (C(23 and 25)), 134.3 (C(9)), 143.1 (C(2)), 143.8 (C(21)), 144.7 (C(12)), 164.4 (C(17)), 168.7 (C(8)); *m/z* (CI) 403 ([M+H]$^+$, 100%); HRMS calcd. for $C_{23}H_{23}N_4OS$ [M+H]$^+$ 403.1587, found: 403.1566.

(*S*)-*N*-(2-aminophenyl)-4-(((4-(4-hydroxyphenyl)-4,5-dihydrothiazol-2-yl)amino)methyl)benzamide (59e)



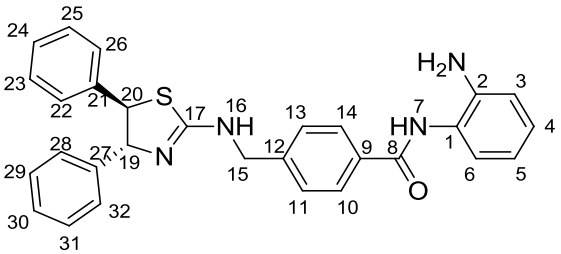
A modified procedure from Kim and Cha was followed.[158] To a solution of thiourea (**79e**) (0.35 g, 0.652 mmol) in anh. THF (10 mL) was added triphenylphosphine (0.257 g, 0.978 mmol) and then a solution of diethyl azodicarboxylate (0.154 ml, 0.978 mmol) in anh. THF (5 mL) dropwise by syringe over 5 min. The solution was stirred for 75 min at rt then HCl in diethylether (2M, 5 mL) was added and stirred for 5 h at rt. The suspension was placed in a refrigerator for 3 days, then filtered and the hygroscopic white solid was partitioned between 5% methanol/dichloromethane (25 mL) and sat. aq. sodium hydrogen carbonate (20 mL). The aqueous layer was extracted with 5% methanol/dichloromethane (2 × 25 mL), then the combined organics were washed with brine (20 mL), dried Na_2SO_4 and evaporated. Purification by column chromatography on silica gel (8% methanol/dichloromethane) gave **59e** as a white solid (0.128 g, 47%), mp 128-131 °C; $[\alpha]_D^{20}$ -63.8 (*c* 1.0, MeOH); ν_{max} (cm^{-1}) 3250 (NH), 1607 (C=O); ^{1}H NMR (400 MHz, methanol-d_4) δ ppm 3.15 (1H, dd, *J*=10.5, 7.5 Hz, H(20)), 3.66 (1H, dd, *J*=10.7, 7.7 Hz, H(20)'), 4.49 - 4.56 (1H, m, H(15)), 4.56 - 4.66 (1H, m, H(15)'), 5.21 (1H, t, *J*=7.4 Hz, H(19)), 6.74 (2H, d, *J*=8.5 Hz, H(23 and 25)), 6.80 (1H, t, *J*=7.5 Hz, H(5)), 6.93 (1H, d, *J*=7.8 Hz, H(3)), 7.03 - 7.16 (3H, m, H(4) and H(22 and 26)), 7.21 (1H, d, *J*=7.8 Hz, H(6)), 7.50 (2H, d, *J*=8.2 Hz, H(11 and 13)), 7.98 (2H, d, *J*=8.2 Hz, H(10 and 14)); ^{13}C NMR (150 MHz, DMSO-d_6) δ ppm 42.0 (C(15)), 47.1 (C(20)), 74.0 (C(19)), 114.8 (C(23 and 25)), 116.2 (C(3)), 116.4 (C(5)), 123.3 (C(1)), 126.5 (C(4)), 126.8 (C(6)), 127.1 (C(11 and 13)), 127.3 (C(10 and 14)), 127.8 (C(22 and 26)), 133.0 (C(9)), 134.2 (C(21)), 143.2 (C(2)), 143.5 (C(12)), 156.3 (C(24)), 159.8 (C(17)), 165.2 (C(8)); *m/z* (CI) 419 ($[M+H]^+$, 85%); HRMS calcd. for $C_{23}H_{23}N_4O_2S$ $[M+H]^+$ 419.1536, found: 419.1525.

***N*-(2-aminophenyl)-4-((((4*S*,5*S*)-4,5-diphenyl-4,5-dihydrothiazol-2-yl)amino)methyl)benzamide ((S,S)-59f)**



Thiourea **(R,S)-79f** (0.572 g, 0.96 mmol) was reacted according to general procedure B for 10 min. The resulting cream solid was recrystallized twice from ethyl acetate and hexane to give **(S,S)-59f** as a white solid (0.151 g, 33%), mp 162-164 °C; $[\alpha]_D^{20}$ -79.3 (*c* 1.0, MeOH); ν_{max} (cm^{-1}) 3239 (NH), 1610 (C=O); ^{1}H NMR (600 MHz, chloroform-*d*) δ ppm 4.31 (2H, br s, NH_2), 4.50 (2H, s, H(15) and H(15)'), 4.75 (1H, d, *J*=6.8 Hz, H(20)), 5.25 (1H, d, *J*=6.8 Hz, H(19)), 6.77 - 6.85 (2H, m, ArH), 7.08 (1H, t, *J*=7.7 Hz, ArH), 7.10 - 7.16 (2H, m, ArH), 7.20 - 7.34 (9H, m, ArH), 7.36 (2H, d, *J*=7.9 Hz, H(11 and 13)), 7.82 (2H, d, *J*=7.9 Hz, H(10 and 14)), 8.17 (1H, br s, H(7)); ^{13}C NMR (150 MHz, chloroform-*d*) δ ppm 49.3 (C(15)), 64.0 (C(19)), 82.6 (C(20)), 118.5 (C(3)), 119.8 (C(6)), 124.6 (C(1)), 125.5 (C(4)), 126.5 (C(28 and 32)), 127.4 (C(5)), 127.8 (C(30) and C(29 and 31), 127.8 (C(11 and 13)), 127.9 (C(22 and 26)), 128.1 (C(24)), 128.6 (C(10 and 14)), 128.9 (C(23 and 25)), 133.2 (C(9)), 140.1 (C(27)), 140.9 (C(2)), 142.0 (C(21)), 143.2 (C(12)), 161.5 (C(17)), 165.8 (C(8)); *m/z* (ES, %) 477 ([M-H]$^-$, 100); HRMS calcd. for $C_{29}H_{26}N_4OS$ [M-H]$^-$ 477.1749, found: 477.1761.

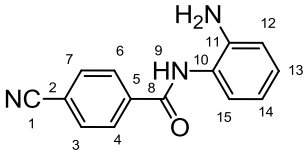
***N*-(2-aminophenyl)-4-((((4*R*,5*R*)-4,5-diphenyl-4,5-dihydrothiazol-2-yl)amino)methyl)benzamide ((R,R)-59f)**



Thiourea **(S,R)-79f** (0.570 g, 0.96 mmol) was reacted according to general procedure B for 10 min. The resulting cream solid was recrystallized from ethyl acetate and hexane to give **(S,S)-59f** as a white solid (0.165 g, 36%), mp 166-168 °C; $[\alpha]_D^{20}$ +80.3 (*c* 1.0, MeOH); ν_{max} (cm^{-1}) 3272 (NH), 1610 (C=O); ^{1}H NMR (600 MHz, chloroform-*d*) δ ppm 3.87 (2H, br s, NH_2), 4.49 (2H, s, H(15) and H(15)'), 4.75 (1H, d,

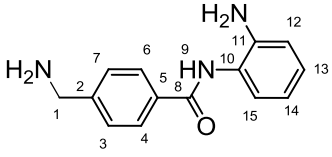
J=6.7 Hz, H(20)), 5.24 (1H, d, *J*=6.7 Hz, H(19)), 6.77 - 6.85 (2H, m, ArH), 7.08 (1H, t, *J*=7.7 Hz, ArH), 7.10 - 7.17 (2H, m, ArH), 7.18 - 7.40 (11H, m, ArH), 7.80 (2H, d, *J*=7.5 Hz, H(10 and 14)), 8.20 (1H, br s, H(7)); ^{13}C NMR (150 MHz, chloroform-*d*) δ ppm 49.2 (C(15)), 64.0 (C(19)), 82.7 (C(20)), 118.4 (C(3)), 119.8 (C(6)), 124.7 (C(1)), 125.5 (C(4)), 126.5 (C(28 and 32)), 127.3 (C(5)), 127.7 (C(30)), 127.7 (C(29 and 31)), 127.8 (C(11 and 13)), 127.9 (C(22 and 26)), 128.0 (C(24)), 128.5 (C(10 and 14)), 128.9 (C(23 and 25)), 133.2 (C(9)), 140.2 (C(27)), 140.9 (C(2)), 142.1 (C(21)), 143.3 (C(12)), 161.3 (C(17)), 165.9 (C(8)); *m/z* (ES, %) 477 ([M-H]$^-$, 100); HRMS calcd. for $C_{29}H_{26}N_4OS$ [M-H]$^-$ 477.1749, found: 477.1770.

4-cyano-*N*-(2-aminophenyl)benzamide (60)[107]



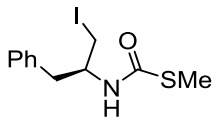
To a solution of 4-cyanobenzoic acid (0.294 g, 2.0 mmol, 1 eq) in DMF (8 ml) was added EDC (0.767 g, 4.0 mmol, 2 eq), HOBT (0.324 g, 2.4 mmol, 1.2 eq), triethylamine (1.12 ml, 8.0 mmol, 4 eq) and phenylene-1,2-diamine (0.432 g, 4.0 mmol, 2 eq). The resulting solution was stirred at rt for 20 hours. Then diluted with brine (400 ml) and filtered, the solids were washed with water and then dried *in vacuo* to give a **60** as a beige solid (0.272 g, 1.12 mmol, 56%), mp 204-205 °C; ν_{max} (cm^{-1}) 3411 (NH), 3355 (NH), 3270 (NH), 2238 (C≡N), 1642 (C=O); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 4.95 (2H, br s, NH_2), 6.58 (1H, t, *J*=7.4 Hz, H(14)), 6.77 (1H, d, *J*=7.9 Hz, H(12)), 6.98 (1H, t, *J*=7.4 Hz, H(13)), 7.14 (1H, d, *J*=7.6 Hz, H(15)), 7.99 (2H, d, *J*=8.0 Hz, H(4 and 6)), 8.12 (2H, d, *J*=8.0 Hz, H(3 and 7)), 9.86 (1H, br s, H(9)); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 113.6 (C(2)), 116.0 (C(12)), 116.1 (C(14)), 118.4 (C(1)), 122.5 (C(10)), 126.9 (C(7)), 126.9 (C(13)), 128.7 (C(4 and 6)), 132.3 (C(3 and 7)), 138.7 (C(5)), 143.4 (C(11)), 164.1 (C(8)); *m/z* (EI$^+$) 237 (M^+, 38%), 130 ([CNC_6H_4CO]$^+$, 38%), 107 ([$C_6H_4NH_2NH$]$^+$, 100%); HRMS $C_{14}H_{11}N_3O^+$ requires: 237.0897, found: 237.0905.

4-(aminomethyl)-*N*-(2-aminophenyl)benzamide (61)



To a solution of 4-cyano-*N*-(2-aminophenyl)benzamide **60** (0.25 g, 1.05 mmol) in methanol (40 ml) was added H_2SO_4 (0.114 ml, 2.1 mmol, 2 eq) and 10% activated Pd on carbon (0.2 g). The flask was evacuated and filled with nitrogen three times to ensure there was no oxygen in the flask, then evacuated and filled with hydrogen. Stirred at rt for 5.5 h under a hydrogen balloon. Then filtered through celite washing with methanol [caution do not let the celite with Pd become dry fire hazard!]. The filtrate was neutralised with 1M sodium hydroxide in methanol (2 ml). The resulting mixture was filtered and the filtrate evaporated to give a pink oil (0.23 g) which was purified by column chromatography on silica gel (1% ammonia (30% aq.), 14% methanol, 85% chloroform) to give **61** as a white solid (0.156 g, 0.65 mmol, 62 %), mp 189-190 °C; ν_{max} (cm^{-1}) 3260 (NH), 3224 (NH), 1635 (C=O); ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.90 (2H, br s, CH_2NH_2), 3.77 (2H, s, CH_2), 4.88 (2H, br s, $ArNH_2$), 6.59 (3H, t, *J*=7.5 Hz, 5-anilyl), 6.77 (1H, d, *J*=7.2 Hz, 3-anilyl), 6.96 (1H, t, *J*=7.5 Hz, 4-anilyl), 7.15 (1H, d, *J*=7.5 Hz, 6-anilyl), 7.44 (2H, d, *J*=8.3 Hz, 3,5-aryl), 7.91 (2H, d, *J*=8.3 Hz, 2,6-aryl), 9.61 (1H, br s, CONH); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 45.4 (C(1)), 116.2 (C(12)), 116.3 (C(14)), 123.5 (C(10)), 126.4 (C(15)), 126.7 (C(13)), 126.7 (C(4 and 6)), 127.6 (C(3 and 7)), 132.5 (C(5)), 143.1 (C(11)), 148.0 (C(2)), 165.2 (C=O); *m/z* (EI^+) 241 (M^+, 51%), 134 ([M-C6H$_4$NH$_2$NH]$^+$, 100%); HRMS $C_{14}H_{15}N_3O^+$ requires: 241.1210, found: 241.1213.

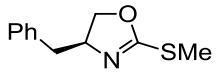
_N_-[(2_S_)-1-iodo-3-phenylpropan-2-yl](methylsulfanyl)formamide (64a)



To a solution of oxazolidine-2-thione **48a** (1.5 g, 7.76 mmol, 1 eq) in acetone (22 ml) was added iodomethane (0.72 ml, 11.6 mmol, 1.5 eq). The solution was stirred at reflux for 2 h then evaporated and partitioned between sat. aq. sodium hydrogen carbonate (30 ml) and dichloromethane (50 ml), the aqueous layer was extracted with dichloromethane (50 ml) then the combined organics were washed with brine, dried $MgSO_4$, and filtered and evaporated to give iodide **64a** as a white solid (2.41 g, 7.19 mmol, 93%), mp 91-93 °C; ν_{max} (cm^{-1}) 3284 (NH), 1640 (C=O); ^{1}H NMR (500 MHz, chloroform-*d*) δ ppm 2.35 (3H, s, SCH_3), 2.81 (1H, dd, *J*=13.7, 8.0 Hz, C*H*H'Ph), 2.95 (1H, dd, *J*=13.7, 5.7 Hz, CH*H'*Ph), 3.17 (1H, dd, *J*=10.3, 4.1 Hz, C*H*H'I), 3.39 (1H, dd, *J*=10.2, 4.6 Hz, CH*H'*I), 3.93 (1H, br s, NCH), 5.50 (1H, br s, NH), 7.22 - 7.29 (3H, m, ArH), 7.29 - 7.35 (2H, m, ArH); ^{13}C NMR (125 MHz, chloroform-*d*) δ ppm 12.5

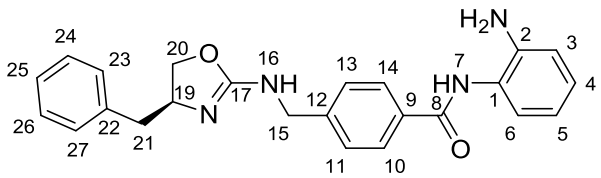
(SCH_3), 12.8 (ICH_2), 40.4 (CH_2Ph), 51.8 (CH), 127.2, 128.9, 129.3, 136.6 (4 ArC), 167.6 (C=O); *m/z* (CI^+) 336 ($[M+H]^+$, 10%), 244 ($[M\text{-}PhCH_2]^-$, 70%); HRMS $C_{11}H_{15}NOSI^+$ requires: 335.9919, found: 335.9911.

(4*S*)-4-benzyl-2-(methylsulfanyl)-4,5-dihydro-1,3-oxazole (65a)



To a solution of iodide **64a** (1.8 g, 5.37 mmol) in acetonitrile (20 ml) was added potassium carbonate (1.33 g, 9.62 mmol, 1.8 eq) and stirred at reflux for 5 h. The mixture was then evaporated, dissolved/suspended in EtOAC (30 ml) and washed with water (20 ml), the aqueous layer was extracted with EtOAC (20 ml). The combined organics were washed with brine and dried Na_2SO_4 and evaporated to give a yellow oil (1.12 g). Purification by column chromatography on silica gel (hexane:ethyl acetate, 4:1) gave **65a** as a pale yellow oil (1.00 g, 4.82 mmol, 90%); ν_{max} (cm^{-1}) 1747 (C=N); ^{1}H NMR (500 MHz, chloroform-*d*) δ ppm 2.48 (3H, s, CH_3), 2.66 (1H, dd, *J*=13.6, 8.8 Hz, C*H*H'Ph), 3.14 (1H, dd, *J*=13.7, 4.9 Hz, CH*H'*Ph), 4.09 (1H, dd, *J*=8.0, 7.1 Hz, C*H*H'O), 4.28 (1H, t, *J*=8.7 Hz, CH*H'*O), 4.36 - 4.46 (1H, m, CHN), 7.18 - 7.25 (3H, m, ArH), 7.30 (15H, t, *J*=7.6 Hz, ArH); ^{13}C NMR (125 MHz, chloroform-*d*) δ ppm 14.6 (SCH_3), 41.8 (CH_2Ph), 67.8 (CHN), 73.7 (CH_2O), 126.7, 128.6, 129.3, 137.8 (4 ArC), 166.7 (C=N); *m/z* (EI^+) 207 (M^+, 12%), 116 ($[M\text{-}CH_2Ph]^+$, 100%); HRMS $C_{11}H_{13}NOS^+$ requires: 207.0712, found: 207.0716.

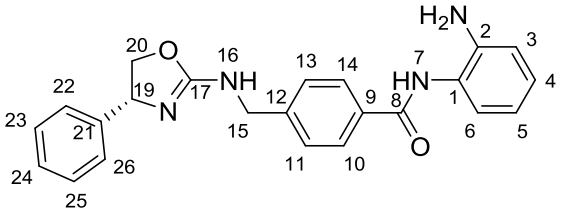
(*S*)-*N*-(2-aminophenyl)-4-(((4-benzyl-4,5-dihydrooxazol-2-yl)amino)methyl)benzamide (66a)



To a solution of **(*S*)-80a** (0.44 g, 0.88 mmol) in dichloromethane (3.4 mL) was added trifluoroacetic acid (0.6 mL). The solution was stirred at rt for 5 h, then trifluoroacetic acid (0.2 mL) was added and the solution stirred at rt for a further 30 min. The reaction was diluted with dichloromethane (50 mL) and sodium bicarbonate (sat. aq., 30 mL) then stirred vigorously for 5 min. The mixture was separated and the organic layer washed with brine (30 mL), dried Na_2SO_4 and evaporated. Purification by column chromatography on silica gel (1% ammonia (30% aq.), 1% methanol, 98% ethyl

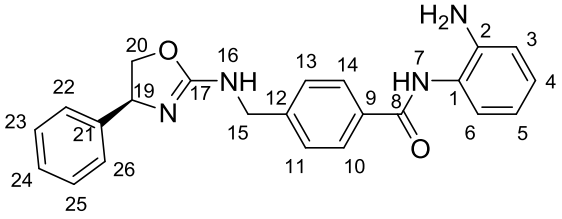
acetate) gave **66a** as a white solid (0.167 g, 0.416 mmol, 47%), mp 146-147 °C; $[\alpha]_D^{25}$ +8.8 (*c* 0.5, methanol); ν_{max} (cm^{-1}) 3202 (NH), 1674 (C=N), 1647 (C=O); ^{1}H NMR (500 MHz, chloroform-*d*) δ ppm 2.62 (1H, dd, *J*=13.5, 8.3 Hz, H(21)), 3.00 (1H, dd, *J*=13.5, 5.0 Hz, H(21)'), 3.87 (2H, br s, NH_2), 3.98 (1H, t, *J*=7.7 Hz, H(20), 4.18 (1H, t, *J*=8.1 Hz, H(20)'), 4.22 - 4.30 (1H, m, H(19)), 4.32 - 4.44 (2H, m, H(15) and H(15)'), 6.74 - 6.85 (2H, m, H(5) and H(3)), 7.07 (1H, t, *J*=7.3 Hz, H(4)), 7.15 (2H, d, *J*=7.1 Hz, ArH), 7.20 (1H, t, *J*=6.9 Hz, ArH), 7.23 - 7.36 (5H, m, ArH), 7.81 (2H, d, *J*=7.4 Hz, H(10 and 14)), 8.22 (1H, br s, H(7)); *m/z* (CI) 401 ($[M+H]^+$, 100%); HRMS $C_{24}H_{25}N_4O_2^+$ requires: 401.1972, found: 401.1963.

(*R*)-*N*-(2-aminophenyl)-4-(((4-phenyl-4,5-dihydrooxazol-2-yl)amino)methyl)benzamide ((*R*)-66b)



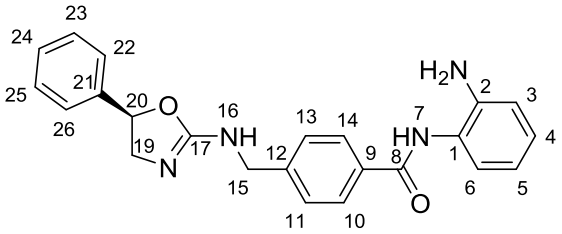
To a solution of **(*R*)-80b** (150 mg, 0.308 mmol) in dichloromethane (5 mL) was added TFA (0.47 mL, 6.16 mmol) dropwise then stirred at rt for 3 h. Quenched with sodium bicarbonate (sat. aq., 20 mL) and extracted with ethyl acetate (2 × 30 mL), the combined organic layers were washed with brine (30 mL), dried Na_2SO_4 and evaporated to give a white solid that was purified by column chromatography on silica gel (1% ammonia (30% aq.), 1% methanol, 98% ethyl acetate) to give **(*R*)-66b** as a white solid (38 mg, 0.098 mmol, 32%), mp 115-118 °C; $[\alpha]_D^{25}$ -14.0 (*c* 1.0, methanol); ν_{max} (cm^{-1}) 3287 (NH), 1649 (C=O); ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 4.04 (1H, t, *J*=7.5 Hz, H(20)), 4.11 (1H, br s, NH_2), 4.33 (2H, s, H(15) and H(15)'), 4.56 (1H, t, *J*=8.6 Hz, H(20)'), 5.02 (1H, dd, *J*=8.8, 7.3 Hz, H(19)), 6.68 – 6.61 (2H, m, H(5) and H(3)), 7.04 (1H, td, *J*=7.6, 1.0 Hz, H(4)), 7.12 - 7.36 (8H, m, ArH), 7.73 (2H, d, *J*=7.9 Hz, H(10 and 14)), 8.52 (1H, br s, H(7)); ^{13}C NMR (75 MHz, chloroform-*d*) δ ppm 46.5 (C(15)), 67.4 (C(19)), 75.5 (C(20)), 118.3 (C(3)), 119.6 (C(5)), 124.6 (C(1)), 125.8 (C(4)), 126.6 (C(11 and 13)), 127.4 (C(6) and C(22 and 26), 127.6 (C(24)), 127.9 (C(23 and 25)), 128.8 (C(10 and 14)), 133.1 (C(9)), 141.2 (C(21)), 143.2 (C(12)), 143.6 (C(2)), 161.9 (C(17)), 166.1 (C(8)); *m/z* (ESI^+) 387 ($[M+H]^+$, 100%); HRMS $C_{23}H_{23}N_4O_2^+$ requires: 387.1816, found: 387.1808.

(*S*)-*N*-(2-aminophenyl)-4-(((4-phenyl-4,5-dihydrooxazol-2-yl)amino)methyl)benzamide ((*S*)-66b)



To a solution of **(*S*)-80b** (103 mg, 0.21 mmol) in dichloromethane (0.9 mL) was added trifluoroacetic acid (0.1 mL). The solution was stirred at rt for 1.5 h, then trifluoroacetic acid (0.05 mL) was added and the solution stirred at rt for a further 2.5 h. The reaction was diluted with dichloromethane (10 mL) and sodium bicarbonate (sat. aq., 10 mL) then stirred vigorously for 5 min. The mixture was separated and the organic layer washed with sodium bicarbonate (sat. aq., 10 mL), dried Na_2SO_4 and evaporated. Purification by column chromatography on silica gel (1% ammonia (30% aq.), 1% methanol, 98% ethyl acetate) gave **(*S*)-66b** as a white solid (60 mg, 0.155 mmol, 74%), mp 85-86 °C; $[\alpha]_D^{25}$ +12.2 (*c* 0.5, methanol); ν_{max} (cm^{-1}) 3254 (NH), 1649 (C=O); ^{1}H NMR (500 MHz, chloroform-*d*) δ ppm 3.91 (2H, br s, NH_2), 4.09 (1H, t, *J*=7.6 Hz, H(20)), 4.42 (2H, br s, H(15) and H(15)'), 4.62 (1H, t, *J*=8.5 Hz, H(20)'), 5.09 (1H, t, *J*=7.9 Hz, H(19)), 6.75 - 6.88 (2H, m, H(5) and H(3)), 7.09 (1H, t, *J*=7.6 Hz, H(4)), 7.20 - 7.38 (8H, m, ArH), 7.80 (2H, d, *J*=6.3 Hz, H(10 and 14)), 8.32 (1H, br s, H(7)); ^{13}C NMR (125 MHz, chloroform-*d*) δ ppm 46.9 (C(15)), 67.9 (C(19)), 75.7 (C(20)), 118.7 (C(3)), 120.0 (C(5)), 125.0 (C(1)), 125.8 (C(4)), 126.8 (C(11 and 13)), 127.6 (C(6)), 127.7 (C(22 and 26)), 127.8 (C(24)), 128.1 (C(23 and 25)), 129.0 (C(10 and 14)), 133.5 (C(9)), 141.3 (C(21)), 143.6 (C(12)), 144.0 (C(2)), 162.1 (C(17)), 166.2 (C(8)); *m/z* (ESI^-) 385 ($[M-H]^-$, 100%); HRMS $C_{23}H_{21}N_4O_2^-$ requires: 385.1670, found:385.1673.

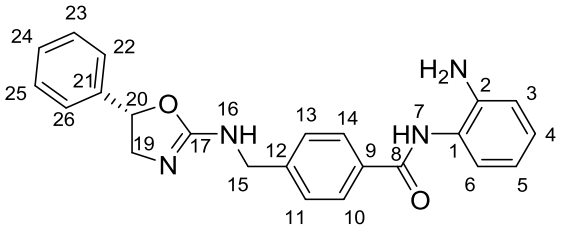
(*R*)-*N*-(2-aminophenyl)-4-(((5-phenyl-4,5-dihydrooxazol-2-yl)amino)methyl)benzamide ((*R*)-66d)



To a solution of **(*R*)-80d** (280 mg, 0.575 mmol) in dichloromethane (4 mL) was added trifluoroacetic acid (0.6 mL, 7.79 mmol) dropwise. The resulting solution was

stirred at rt for 3.5 h then a further 0.4 mL of trifluoroacetic acid was added and stirred for another 1 h. The reaction was quenched with sodium bicarbonate (sat. aq., 15 mL) and then extracted with ethyl acetate (3 × 20 mL). The combined organics were washed with brine, dried Na_2SO_4 and evaporated to give a white solid (195 mg) which was purified by flash column chromatography on silica gel (1.6-8% (2M ammonia/methanol) in dichloromethane) to give a white solid (140 mg). Further purification by MDAP gave **(*R*)-66d** (75 mg, 0.194 mmol, 34%) as a white solid, mp 196-197 °C; $[\alpha]_D^{25}$ -23.0 (*c* 1.0, methanol); ν_{max} (cm^{-1}) 3398 (NH), 3238 (NH), 3340 (NH), 1668 (C=N), 1641 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 3.44 (1H, dd, *J*=12.2, 6.6 Hz, H(19), 4.04 (1H, dd, *J*=12.2, 9.2 Hz, H(19)'), 4.38 (2H, s, H(15) and H(15)'), 4.90 (2H, br s, NH_2), 5.51 (1H, dd, *J*=9.2, 6.6 Hz, H(20)), 6.62 (1H, t, *J*=7.3 Hz, H(5)), 6.80 (1H, d, *J*=7.8 Hz, H(3)), 6.98 (1H, t, *J*=7.3 Hz, H(4), 7.10 - 7.24 (2H, m, H(6) and H(16)), 7.26 - 7.36 (3H, m, H(22 and 26) and H(24)),7.40 (2H, t, J=7.1 Hz, H(23 and 25)), 7.46 (2H, d, *J*=8.0 Hz, H(11 and 13)), 7.96 (2H, d, *J*=8.0 Hz, H(10 and 14)), 9.64 (1H, s, H(7)); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 46.1 (C(15)), 61.2 (C(19)), 80.3 (C(20)), 116.6 (C(3)), 116.8 (C(5)), 123.9 (C(1)), 125.9 (C(22 and 26)), 126.9 (C(4)), 127.1 (C(6)), 127.3 (C(11 and 13)), 128.2 (C(10 and 14)), 128.3 (C(24)), 129.1 (C(23 and 25)), 133.5 (C(9)), 142.2 (C(21)), 143.6 (C(12)), 144.2 (C(2)), 160.5 (C(17)), 165.7 (C(8)); *m/z* (ESI^+) 387 ($[M+H]^+$, 100%); HRMS $C_{23}H_{23}N_4O_2^+$ requires: 387.1816, found: 387.1813.

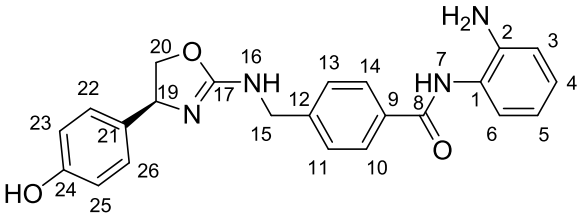
(*S*)-*N*-(2-aminophenyl)-4-(((5-phenyl-4,5-dihydrooxazol-2-yl)amino)methyl)benzamide ((*S*)-66d)



To a solution of **(*S*)-80d** (0.275 g, 0.565 mmol) in dichloromethane (6 mL) was added trifluoroacetic acid (0.871 mL, 11.30 mmol). The resulting solution was stirred at rt for 3 h, then quenched by pouring onto sodium bicarbonate (sat. aq., 15 mL) and extracted with ethyl acetate (3 × 30 mL). The combined organics were washed with brine, dried Na_2SO_4, and evaporated to give a white solid (240 mg) which was purified by MDAP to give **(*S*)-66d** (75 mg, 0.194 mmol, 34%) as a cream solid, mp 214-215 °C; $[\alpha]_D^{25}$ +22.0 (*c* 0.5, methanol); ν_{max} (cm^{-1}) 3338, (NH), 3237 (NH), 1668 (C=N), 1641

(C=O); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 3.49 (1H, dd, *J*=11.8, 7.0 Hz, H(19)), 4.08 (1H, dd, *J*=11.8, 9.1 Hz, H(19)'), 4.41 (2H, s, H(15) and H(15)'), 4.91 (2H, br s, NH_2), 5.61 (1H, t, *J*=8.0 Hz, H(20)), 6.62 (1H, t, *J*=7.3 Hz, H(5)), 6.80 (1H, d, *J*=7.7 Hz, H(3)), 6.99 (1H, t, *J*=7.0 Hz, H(4)), 7.19 (1H, d, *J*=7.4 Hz, H(6)), 7.31 - 7.38 (3H, m, H(22 and 26) and H(24)), 7.39 - 7.44 (2H, m, H(23 and 25)), 7.46 (2H, d, *J*=8.0 Hz, H(11 and 13)), 7.97 (2H, d, *J*=7.7 Hz, H(10 and 14)), 9.67 (1H, s, H(7)); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 46.0 (C(15)), 59.5 (C(29)), 80.9 (C(20)), 116.6 (C(3)), 116.7 (C(5)), 123.8 (C(1)), 126.1 (C(22 and 26)), 126.9 (C(4)), 127.2 (C(6)), 127.3 (C(11 and 13)), 128.3 (C(10 and 14)), 128.6 (C(24)), 129.1 (C(23 and 25)), 133.7 (C(9)), 141.3 (C(21)), 143.6 (C(12)), 143.6 (C(2)), 160.7 (C(17)), 165.6 (C(8)); *m/z* (ESI$^+$) 387 ([M+H]$^+$, 100%); HRMS $C_{23}H_{23}N_4O_2^+$ requires: 387.1816 , found: 387.1822.

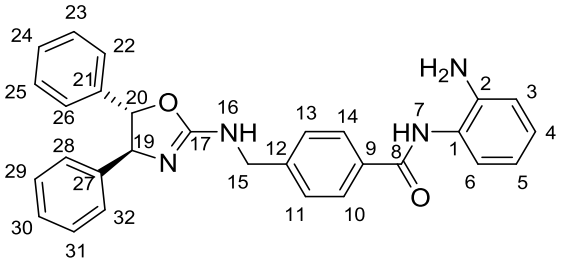
(*S*)-*N*-(2-aminophenyl)-4-(((4-(4-hydroxyphenyl)-4,5-dihydrooxazol-2-yl)amino)methyl)benzamide (66e)



To a solution of **80e** (250 mg, 0.497 mmol) in dichloromethane (6 mL) was added trifluoroacetic acid (0.767 mL, 9.95 mmol). The resulting solution was stirred at rt for 2.5 h, then quenched by pouring onto sodium bicarbonate (sat. aq., 15 mL) and extracted with ethyl acetate (3 × 30 mL). The combined organics were washed with brine, dried Na_2SO_4, and evaporated to give a white solid which was purified by flash column chromatography on silica gel (1-10% (2M ammonia/methanol) in dichloromethane) to give **66e** (141 mg, 0.350 mmol, 70%) as a white solid, mp 158-160 °C; $[\alpha]_D^{25}$ +17.6 (*c* 0.5, methanol); ν_{max} (cm^{-1}) 3262 (NH), 1646 (C=O); ^{1}H NMR (400 MHz, methanol-d_4) δ ppm 4.03 (1H, dd, *J*=8.0, 6.8 Hz, H(20)), 4.39 - 4.54 (2H, m, H(15) and H(15)'), 4.62 (1H, dd, *J*=9.0, 8.0 Hz, H(20)'), 4.99 (1H, dd, *J*=9.0, 6.8 Hz, H(19)), 6.74 (2H, d, *J*=8.6 Hz, H(23 and 25)), 6.79 (1H, td, *J*=7.6, 1.0 Hz, H(5)), 6.92 (1H, dd, *J*=7.8, 1.2 Hz, H(3)), 7.02 - 7.14 (3H, m, H(22 and 26) and H(4)), 7.22 (1H, d, *J*=7.8 Hz, H(6)), 7.52 (2H, d, *J*=8.1 Hz, H(11 and 13)), 7.98 (2H, d, *J*=8.3 Hz, H(10 and 14)); ^{13}C NMR (100 MHz, methanol-d_4) δ ppm 45.6 (C(15)), 66.3 (C(19)), 75.3 (C(20)), 114.9 (C(23 and 25)), 117.4 (C(3)), 118.3 (C(5)), 124.0 (C(1)), 126.3 (C(6)), 127.0 (C(10 and 14)), 127.1 (C(4)), 127.2 (C(11 and 13)), 127.6 (C(22 and 26)), 132.9

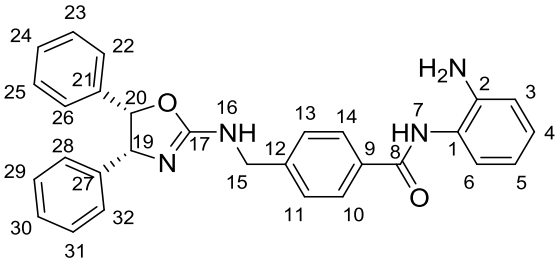
(C(21)), 134.4 (C(9)), 142.3 (C(2)), 143.6 (C(12)), 156.5 (C(24)), 162.5 (C(17)), 167.3 (C(8)); *m/z* (ESI^+) 403 ($[M+H]^+$, 100%); HRMS $C_{23}H_{23}N_4O_3^+$ requires: 403.1765, found: 403.1765.

_N_-(2-aminophenyl)-4-((((4*S*,5*S*)-4,5-diphenyl-4,5-dihydrooxazol-2-yl)amino)methyl)benzamide ((R,R)-66f)



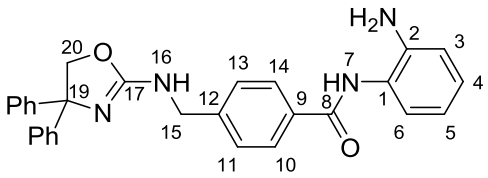
To a solution of **(R,R)-80f** (155 mg, 0.275 mmol) in dichloromethane (2 mL) was added trifluoroacetic acid (0.424 mL, 5.51 mmol) dropwise. The resulting solution was stirred at rt for 2 h, then quenched with sodium bicarbonate (sat. aq., 10 mL) and extracted with ethyl acetate (2 × 15 mL), the combined organics were dried with Na_2SO_4 and evaporated to give a white solid (140 mg) which was purified by flash column chromatography on silica gel (1-6% (2M ammonia/methanol) in dichloromethane) to give **(R,R)-66f** (125 mg, 0.270 mmol, 98%) as a white solid, mp 110-113 °C; $[\alpha]_D^{25}$ -33.2 (*c* 1.0, methanol); ν_{max} (cm^{-1}) 3264 (NH), 1657 (C=O); *m/z* (ESI^+) 463 ($[M+H]^+$, 100%); ^1H NMR (400 MHz, methanol-d_4) δ ppm 4.46 - 4.63 (2H, m, H(15) and H(15)'), 4.89 (1H, m, H(19)), 5.23 (1H, d, *J*=6.6 Hz, H(20)), 6.79 (1H, t, *J*=7.5 Hz, H(5)), 6.92 (1H, dd, *J*=8.1, 1.3 Hz, H(3)), 7.10 (1H, td, *J*=7.7, 1.5 Hz, H(4)), 7.19 - 7.44 (11H, m, ArH), 7.57 (2H, d, *J*=8.3 Hz, H(11 and 13)), 8.02 (2H, d, *J*=8.1 Hz, H(10 and 14)); ^{13}C NMR (100 MHz, methanol-d_4) δ ppm 45.6 (C(15)), 75.9 (C(19)), 89.3 (C(20)), 117.4 (C(3)), 118.3 (C(5)), 124.0 (C(1)), 125.3 (C(11 and 13)), 126.2 (C(28 and 32)), 126.3 (C(4)), 127.1 (C(22 and 26)), 127.2 (C(6)), 127.4 (C(30)), 127.7 (C(29 and 31)), 128.2 (C(24)), 128.4 (C(23 and 25)), 128.5 (C(10 and 14)), 133.0 (C(9)), 139.9 (C(27)), 142.4 (C(21)), 142.8 (C(12)), 143.4 (C(2)), 162.0 (C(17)), 167.3 (C(8)); HRMS $C_{29}H_{27}N_4O_2^+$ requires: 463.2129, found: 463.2128; 94% purity by LCMS.

***N*-(2-aminophenyl)-4-((((4*R*,5*S*)-4,5-diphenyl-4,5-dihydrooxazol-2-yl)amino)methyl)benzamide ((R,S)-66f)**



To a solution of **(R,S)-80f** (414 mg, 0.736 mmol) in dichloromethane (6 mL) was added trifluoroacetic acid (1.134 mL, 14.72 mmol) dropwise. The resulting solution was stirred at rt for 5 h. The reaction was quenched by adding to sodium bicarbonate (sat. aq., 20 mL) and ethyl acetate (50 mL), the layers were separated and the aqueous layer was extracted with another portion of ethyl acetate (20 mL). The combined organics were washed with brine, dried Na_2SO_4 and evaporated to give a white solid (300 mg). Purification by flash column chromatography on silica gel (3-6% (2M ammonia/methanol) in dichloromethane) followed by MDAP gave **(R,S)-66f** (107 mg, 0.231 mmol, 31%) as a white solid, mp 112-113 °C; $[\alpha]_D^{25}$ +51.6 (*c* 0.5, methanol); ν_{max} (cm^{-1}) 3270 (NH), 1652 (C=O); ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 3.90 (2H, br s, NH_2), 4.39 - 4.63 (2H, m, H(15) and H(15)'), 5.38 (1H, d, *J*=8.8 Hz, H(19)), 5.81 (1H, d, *J*=8.8 Hz, H(20)), 6.73 - 6.94 (6H, m, ArH), 6.94 - 7.17 (7H, m, ArH), 7.26 - 7.35 (1H, m, ArH), 7.40 (2H, d, *J*=7.8 Hz, ArH), 7.84 (2H, d, *J*=7.8 Hz, ArH), 8.30 (1H, br s, N(7)); ^{13}C NMR (100 MHz, chloroform-*d*) δ ppm 46.5 (C(15)), 72.0 (C(19)), 85.7 (C(20)), 118.3 (C(3)), 119.7 (C(5)), 124.6 (C(1)), 125.4 (C(4)), 126.2 (C(11 and 13)), 126.8 (C(6)), 127.2 (C(30)), 127.4 (C(24)), 127.5 (C(28 and 32)), 127.5 (C(22 and 26)), 127.6 (C(29 and 31)), 127.6 (C(23 and 25)), 127.7 (C(10 and 14)), 133.2 (C(9)), 136.4 (C(27)), 139.2 (C(21)), 140.9 (C(12)), 143.3 (C(2)), 161.5 (C(17)), 165.8 (C(8)); *m/z* (ESI^+) 463 ($[M+H]^+$, 100%); HRMS $C_{29}H_{27}N_4O_2^+$ requires: 463.2129, found: 463.2128.

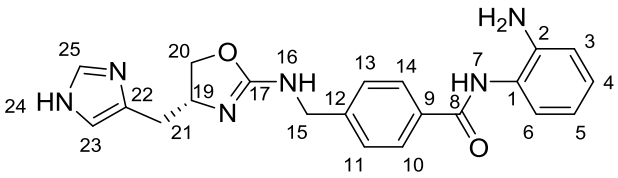
***N*-(2-aminophenyl)-4-(((4,4-diphenyl-4,5-dihydrooxazol-2-yl)amino)methyl)benzamide (66g)**



To a solution of **80g** (280 mg, 0.498 mmol) in dichloromethane (6 mL) was

added trifluoroacetic acid (0.767 mL, 9.95 mmol). The resulting solution was stirred at rt for 2.5 h, then quenched by pouring onto sodium bicarbonate (sat. aq., 15 mL) and extracted with ethyl acetate (3 × 30 mL). The combined organics were washed with brine, dried Na_2SO_4, and evaporated to give a white solid (210 mg) which was purified by flash column chromatography on silica gel (1-6% (2M ammonia/methanol) in dichloromethane) to give **66g** (135 mg, 0.292 mmol, 59%) as a white solid, mp 176-177 °C; ν_{max} (cm^{-1}) 3194 (NH), 1670 (C=N), 1649 (C=O); ^{1}H NMR (400 MHz, methanol-*d*$_4$) δ ppm 4.54 (2H, s, H(15) and H(15)'), 4.79 (2H, s, H(20)), 6.79 (1H, td, *J*=7.6, 0.8 Hz, H(5)), 6.92 (1H, dd, *J*=8.1, 1.2 Hz, H(3)), 7.10 (1H, td, *J*=7.7, 1.3 Hz, H(4)), 7.17 - 7.37 (11H, m, ArH), 7.49 (2H, d, *J*=8.2 Hz, H(11 and 13)), 7.96 (2H, d, *J*=8.2 Hz, H(10 and 14)); ^{13}C NMR (100 MHz, methanol-*d*$_4$) δ ppm 45.4 (C(15)), 76.4 (C(19)), 80.0 (C(20)), 117.4 (C(3)), 118.3 (C(5)), 124.0 (C(1)), 126.3 (C(6)), 126.6 (C(2 × Ph-*ortho*)), 126.6 (C(2 × Ph-*para*)), 127.0 (C(10 and 14)), 127.1 (C(4)), 127.6 (C(11 and 13)), 127.8 (C(2 × Ph-*meta*)), 132.9 (C(9)), 142.4 (C(2)), 143.6 (C(12)), 146.4 (C(2 × Ph-*ipso*)), 161.4 (C(17)), 167.2 (C(8)); *m/z* (ESI^+) 463 ($[M+H]^+$, 100%); HRMS $C_{29}H_{27}N_4O_2^+$ requires: 463.2129, found: 463.2118.

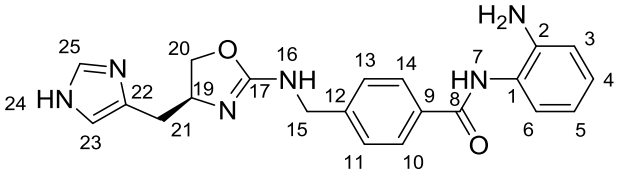
(*R*)-4-(((4-((1*H*-imidazol-4-yl)methyl)-4,5-dihydrooxazol-2-yl)amino)methyl)-*N*-(2-aminophenyl)benzamide ((*R*)-66h)



To a suspension of **(*R*)-80h** (100 mg, 0.204 mmol) in dichloromethane (2 mL) was added trifluoroacetic acid (0.314 mL, 4.08 mmol) dropwise. The resulting solution was stirred at rt for 2 h. Then evaporated and the resulting white solid purified by MDAP to **(*R*)-66h** (43 mg, 0.110 mmol, 54%) as a white solid, mp 191-193 °C; $[\alpha]_D^{25}$ +6.3 (*c* 1.0, methanol); ν_{max} (cm^{-1}) 3227 (NH), 1673 (C=N), 1627 (C=O); ^{1}H NMR (500 MHz, methanol-*d*$_4$) δ ppm 2.74 (1H, dd, *J*=15.5, 5.4 Hz, H(21)), 3.24 (1H, dd, *J*=15.5, 7.3 Hz, H(21)'), 3.88 (1H, dd, *J*=11.3, 5.2 Hz, H(20)), 4.36 (1H, dd, *J*=11.1, 6.7 Hz, H(20)'), 4.43 (2H, s, H(15) and H(15)'), 4.92 - 4.99 (1H, m, H(19)), 6.70 (1H, s, H(23)), 6.79 (1H, t, *J*=7.3 Hz, H(5)), 6.93 (1H, d, *J*=7.7 Hz, H(3)), 7.06 - 7.13 (1H, m, H(4)), 7.21 (1H, d, *J*=7.4 Hz, H(6)), 7.45 (2H, d, *J*=7.7 Hz, H(11 and 13)), 7.59 (1H, s, H25)), 7.97 (2H, d, *J*=8.0 Hz, H(10 and 14)); ^{13}C NMR (125 MHz, methanol-*d*$_4$) δ ppm 28.8 (C(21)), 42.9 (C(15)), 50.6 (C(19)), 54.7 (C(20)), 117.3 (C(3)), 118.3 (C(5)), 118.8

(C(23)), 123.9 (C(1)), 126.2 (C(6)), 126.8 (C(10 and 14)), 127.1 (C(4)), 127.6 (C(11 and 13)), 130.9 (C(25)), 132.8 (C(9)), 134.6 (C(23)), 142.4 (C(12)), 144.2 (C(2)), 158.8 (C(17)), 167.3 (C(8)); *m/z* (ESI^+) 391 ($[M+H]^+$, 100%); HRMS $C_{21}H_{23}N_6O_2^+$ requires: 391.1877, found: 391.1881.

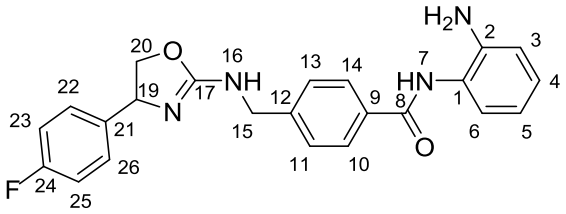
(*S*)-4-(((4-((1*H*-imidazol-4-yl)methyl)-4,5-dihydrooxazol-2-yl)amino)methyl)-*N*-(2-aminophenyl)benzamide ((*S*)-66h)



To a suspension of **(*S*)-80h** (130 mg, 0.265 mmol) in dichloromethane (2 mL) was added trifluoroacetic acid (0.2 mL, 2.60 mmol) dropwise. The resulting solution was stirred at rt for 3 h, then a further 0.2 mL trifluoroacetic acid was added, and stirred at rt for 2 h. Quenched with sodium bicarbonate (sat. aq., 15 mL) and then ethyl acetate (20 mL) was added, but an insoluble white precipitate remained in the aqueous layer. Ethyl acetate was separated and the aqueous layer was basified with 2M NaOH to pH 10/11 then extracted with 20% ethanol/chloroform (4 × 25 mL). The combined organics were washed with brine, dried Na_2SO_4 and evaporated to give a grey solid (40 mg), LCMS showed the product was dissolved in the aqueous layer. The aqueous layer was passed over a 10 g SCX-2 cartridge, then washed with methanol (100 mL), and eluted with 2M ammonia/methanol (100 mL), but LCMS showed the product to be in the flow though and methanol wash fraction. The methanol and water were evaporated to give a white solid which was stirred with 10% methanol/dichloromethane for 30 min, then the inorganics were filtered and the filtrate was evaporated to give a white solid (60 mg) which was purified by MDAP to give **(*S*)-66h** (23 mg, 0.059 mmol, 22%) as a white solid, mp 192-194 ºC; $[\alpha]_D^{25}$ -18.0 (*c* 0.1, methanol); ν_{max} (cm^{-1}) 3241 (NH), 1655 (C=O); ^1H NMR (400 MHz, methanol-d_4) δ ppm 2.74 (1H, ddd, *J*=15.5, 5.5, 1.0 Hz, H(21)), 3.24 (1H, ddd, *J*=15.5, 7.4, 1.0 Hz, H(21)'), 3.87 (1H, dd, *J*=11.3, 5.1 Hz, H(20)), 4.35 (1H, dd, *J*=11.3, 6.8 Hz, H(20)'), 4.43 (2H, s, H(15) and H(15)'), 4.89 - 5.00 (1H, m, H(19)), 6.69 (1H, s, H(23)), 6.79 (1H, t, *J*=7.2 Hz, H(5)), 6.92 (1H, dd, *J*=8.1, 1.2 Hz, H(3)), 7.05 - 7.13 (1H, m, H(4)), 7.20 (1H, d, *J*=7.6 Hz, H(6)), 7.44 (2H, d, *J*=8.3 Hz, H(11 and 13)), 7.57 (1H, s, H(25)), 7.96 (2H, d, *J*=8.1 Hz, H(10 and 14)); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 29.5 (C(21)), 43.1 (C(15)), 50.7 (C(19)), 55.0 (C(20)), 116.6 (C(3)), 116.7 (C(5)), 119.9 (C(23)), 123.8 (C(1)), 126.9 (C(4)), 127.1

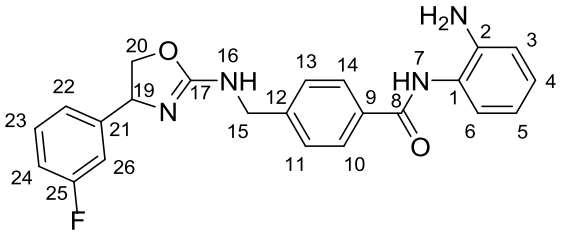
(C(6)), 127.2 (C(11 and 13)), 128.2 (C(10 and 14)), 131.4 (C(25)), 133.4 (C(22)), 134.6 (C(9)), 143.6 (C(2)), 144.9 (C(12)), 157.9 (C(17)), 165.6 (C(8)); *m/z* (ESI^+) 391 ($[M+H]^+$, 100%); HRMS $C_{21}H_{23}N_6O_2^+$ requires: 391.1877, found: 391.1878.

_N_-(2-aminophenyl)-4-(((4-(4-fluorophenyl)-4,5-dihydrooxazol-2-yl)amino)methyl)benzamide (66i)



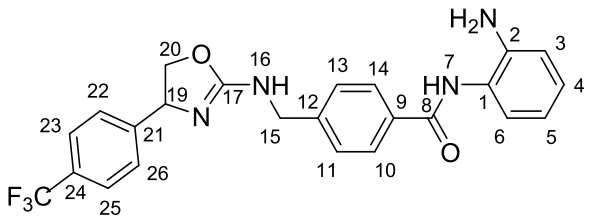
To a solution of **80i** (220 mg, 0.436 mmol) in dichloromethane (6 mL) was added TFA (0.67 mL, 8.72 mmol) dropwise then stirred at rt for 3 h. Quenched with sodium bicarbonate (sat. aq., 10 mL) and extracted with ethyl acetate (2 × 30 mL), the combined organic layers were washed with brine (40 mL), dried Na_2SO_4 and evaporated to give a cream solid (200 mg) that was purified by column chromatography on silica gel (1% ammonia (30% aq.), 1% methanol, 98% ethyl acetate) and again (1% ammonia (30% aq.), 6% methanol, 93% ethyl acetate) to give **66i** as a white solid (108 mg, 0.267 mmol, 61%), mp 104-108 °C; ν_{max} (cm^{-1}) 3245 (NH), 1649 (C=O); ^1H NMR (500 MHz, chloroform-*d*) δ ppm 3.98 - 4.06 (1H, m, H(20)), 4.09 (2H, br s, NH_2), 4.39 (2H, s, H(15) and H(15)'), 4.54 - 4.67 (1H, m, H(20)), 4.97 - 5.14 (1H, m, H(19)), 6.71 - 6.86 (2H, m, ArH), 6.93 - 7.03 (2H, m, ArH), 7.03 - 7.12 (1H, m, ArH), 7.12 - 7.22 (2H, m, ArH), 7.22 - 7.36 (3H, m, ArH), 7.70 - 7.87 (2H, m, ArH), 8.44 (1H, br s, H(7)); ^{13}C NMR (125 MHz, chloroform-*d*) δ ppm 46.4 (C(15)), 66.7 (C(19)), 75.3 (C(20)), 115.3 (d, *J*=22.1 Hz, H(23 and 25), 118.1 (C(3)), 119.5 (C(5)), 124.4 (C(1)), 125.5 (C(4)), 127.2 (C(6) and C(11 and 13)), 127.6 (C(10 and 14)), 127.9 (d, *J*=8.6 Hz, C(22 and 26), 133.0 (C(9)), 139.2 (C(21)), 140.9 (C(12)), 142.9 (C(2)), 162.0 (d, *J*=245.7 Hz, C(24)), 161.7 (C(17)), 165.7 (C(8)); *m/z* (ESI^+) 405 ($[M+H]^+$, 100%); HRMS $C_{23}H_{22}FN_4O_2^+$ requires: 405.1721, found: 405.1710.

_N_-(2-aminophenyl)-4-(((4-(3-fluorophenyl)-4,5-dihydrooxazol-2-yl)amino)methyl)benzamide (66j)



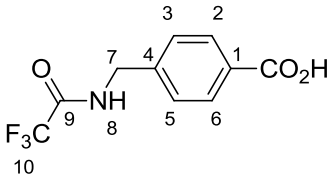
To a solution of **80j** (190 mg, 0.377 mmol) in dichloromethane (3 mL) was added trifluoroacetic acid (0.580 mL, 7.53 mmol) dropwise. The resulting solution was stirred at rt for 2 h, then quenched with sodium bicarbonate (sat. aq., 10 mL) and extracted with ethyl acetate (2 × 20 mL), the combined organics were washed with brine (20 mL) then dried with Na_2SO_4 and evaporated to give a white solid (150 mg) which was purified by flash column chromatography on silica gel (1-6% (2M ammonia/methanol) in dichloromethane) to give **66j** (131 mg, 0.324 mmol, 86%) as a white solid, mp 84-86 °C; ν_{max} (cm^{-1}) 3276 (NH), 1652 (C=O); ^{1}H NMR (400 MHz, methanol-*d*$_4$) δ ppm 4.05 (1H, dd, *J*=8.2, 6.4 Hz, H(20)), 4.41 - 4.56 (2H, m, H(15) and H(15)'), 4.68 (1H, dd, *J*=9.1, 8.3 Hz, H(20)'), 5.09 (1H, dd, *J*=9.0, 6.4 Hz, H(19)), 6.79 (1H, td, J=7.6, 1.0 Hz, H(5)), 6.88 - 7.02 (3H, m, H(3), H(24) and H(26), 7.03 - 7.14 (2H, m, H(4) and H(22)), 7.21 (1H, d, *J*=7.8 Hz, H(6)), 7.33 (1H, td, *J*=7.9, 5.9 Hz, H(23)), 7.53 (2H, d, *J*=8.1 Hz, H(11 and 13)), 8.00 (2H, d, *J*=8.1 Hz, H(10 and 14)); ^{13}C NMR (100 MHz, methanol-*d*$_4$) δ ppm 45.6 (15), 66.2 (19), 74.9 (20), 112.7 (d, *J*=22.4 Hz, C(24)), 113.7 (d, *J*=20.8 Hz, C(26)), 117.3 (C(3)), 118.3 (C(5)), 121.9 (d, *J*=3.2 Hz, C(22)), 124.0 (C(1)), 126.3 (C(6)), 127.0 (C(11 and 13)), 127.2 (C(4)), 127.7 (C(10 and 14)), 130.0 (d, *J*=8.0 Hz, C(23)), 133.0 (C(9)), 142.4 (C(2)), 143.4 (C(12)), 146.8 (d, *J*=7.2 Hz, C(21)), 163.0 (d, *J*=245.3 Hz, C(25)), 163.0 (C(17)), 167.2 (C(8)); *m/z* (ESI^+) 405 ($[M+H]^+$, 100%); HRMS $C_{23}H_{22}FN_4O_2^+$ requires: 405.1721, found: 405.1723; 94% purity by LCMS.

_N_-(2-aminophenyl)-4-(((4-(4-(trifluoromethyl)phenyl)-4,5-dihydrooxazol-2-yl)amino)methyl)benzamide (66k)



To a solution of **80k** (190 mg, 0.343 mmol) in dichloromethane (4 mL) was added trifluoroacetic acid (0.528 mL, 6.85 mmol). The resulting solution was stirred at rt for 2.5 h, then quenched by pouring onto sodium bicarbonate (sat. aq., 15 mL) and extracted with ethyl acetate (3 × 30 mL). The combined organics were washed with brine, dried Na_2SO_4, and evaporated to give a white solid (130 mg) which was purified by flash column chromatography on silica gel (1-6% (2M ammonia/methanol) in dichloromethane) to give **66k** (110 mg, 0.242 mmol, 71%) as a white solid, mp 198-199 °C; ν_{max} (cm^{-1}) 3265 (NH), 1653 (C=O); ^{1}H NMR (500 MHz, methanol-d_4) δ ppm 4.08 (1H, dd, *J*=8.2, 6.6 Hz, H(20)), 4.44 - 4.58 (2H, m, H(15) and H(15)'), 4.73 (1H, dd, *J*=9.2, 8.2 Hz, H(20)'), 5.18 (1H, dd, *J*=9.2, 6.6 Hz, H(19)), 6.80 (1H, t, *J*=7.4 Hz, H(5)), 6.93 (1H, d, *J*=8.0 Hz, H(3)), 7.11 (1H, t, *J*=7.1 Hz, H(4)), 7.22 (1H, d, *J*=7.7 Hz, H(6)), 7.43 (2H, d, *J*=8.0 Hz, H(22 and 26), 7.54 (2H, d, *J*=8.0 Hz, H(11 and 13)), 7.64 (2H, d, *J*=8.0 Hz, H(23 and 25)), 8.01 (2H, d, *J*=8.0 Hz, H(10 and 14)); ^{13}C NMR (125 MHz, methanol-d_4) δ ppm 45.6 (C(15)), 66.3 (C(19)), 74.8 (C(20)), 117.4 (C(3)), 118.3 (C(5)), 124.3 (q, *J*=271.0 Hz, CF_3), 124.0 (C(23)), 125.1 (q, *J*=3.9 Hz, C(23 and 25)), 126.3 (C(6)), 126.7 (C(22 and 26)), 127.0 (C(10 and 14)), 127.2 (C(4)), 127.7 (C(11 and 13)), 129.2 (q, *J*=32.4 Hz, C(24)), 133.0 (C(9)), 142.4 (C(2)), 143.4 (C(12)), 148.3 (C(21)), 163.2 (C(17)), 167.2 (C(8)); *m/z* (ESI^+) 455 ($[M+H]^+$, 100%); HRMS $C_{24}H_{22}F_3N_4O_2^+$ requires: 455.1689, found: 455.1686; 92% purity by LCMS.

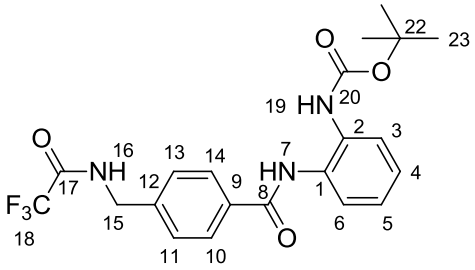
4-[(trifluoroacetamido)methyl]benzoic acid (68)[108]



Trifluoracetic anhydride (1.74 ml, 12.5 mmol, 2.5 eq) was added dropwise to 4-(aminomethyl)benzoic acid (0.756 g, 5.0 mmol, 1.0 eq) at 0 °C. Upon completion of addition the mixture was homogeneous. Stirring continued for 2 h at rt, then ice-water was added to precipitate the product which was filtered, washed with water and dried to give **68** as a white powder (1.06 g, 4.25 mmol, 85%), mp 212-213 °C (lit. 199-203 °C)[108]; ν_{max} (cm^{-1}) 3291 (NH), 1708 (C=O), 1684 (C=O); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 4.45 (2H, d, *J*=6.0 Hz, C(7)H_2), 7.38 (2H, d, *J*=8.4 Hz, H(3 and 5)), 7.92 (2H, d, *J*=8.4 Hz, H(2 and 6)), 10.07 (1H, t, *J*=6.0 Hz, H(8)), 12.93 (1H, br s, CO_2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 42.3 (C(7)), 116.0 (q, *J*=288.0 Hz, C(10)), 127.4 (C(3 and 5)), 129.6 (C(2 and 6)), 129.8 (C(1)), 142.4 (C(4)), 156.5 (q, *J*=36.2 Hz, C(9)),

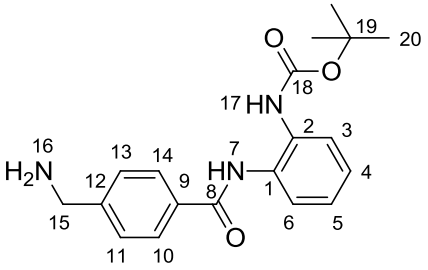
167.1 (CO_2H).

tert-butyl _N_-(2-{4-[(trifluoroacetamido)methyl]benzamido}phenyl)carbamate (69)[109]



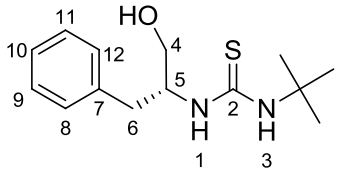
To a suspension of benzoic acid **68** (1.0 g, 4.05 mmol, 1.1 eq) in dichloromethane (7 ml) was added dropwise oxalyl chloride (0.467 ml, 5.52 mmol, 1.5 eq), followed by two drops of DMF. Stirred at rt until bubbles stopped forming, and then at 40 °C for 1 h. Solvent evaporated and then co-evaporated with toluene. Then dissolved in dichloromethane (3 ml) and added dropwise to a solution of **39** in dichloromethane (3 ml) and pyridine (6 ml) at 0 °C. Stirred at 0 °C for 30 min then warmed to rt and stirred for a further 20 h. To the reaction mixture was added sodium hydrogen carbonate (sat. aq. 15 ml) and then extracted with chloroform (2 × 30 ml), the combined organic layer was washed with brine (10 ml), dried $MgSO_4$ and evaporated to give **69** as a pale yellow solid (1.522 g, 3.48 mmol, 95%), mp 157-158 °C; ν_{max} (cm^{-1}) 3334 (NH), 1722 (C=O), 1672 (C=O), 1663 (C=O); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 1.43 (9H, s, (3 × C(23)H_3), 4.47 (2H, br s, C(15)H_2), 7.09 - 7.23 (2H, m, H(4) and H(5)), 7.43 (2H, d, *J*=8.0 Hz, H(11 and 13), 7.48 - 7.56 (2H, m, H(3) and H(6)), 7.93 (2H, d, *J*=8.0 Hz, H(10 and 14)), 8.66 (1H, br s, H(19)), 9.82 (1H, br s, H(7)), 10.10 (1H, br s, H(16)); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 28.0 (C(23)), 42.3 (C(15)), 79.6 (C(22)), 116.0 (q, *J*=288.0 Hz, C(17)), 123.8 (C(3)), 124.1 (C(6)), 125.6 (C(4)), 126.0 (C(5)), 127.4 (C(11 and 13)), 127.9 (C(10 and 14)), 129.7 (C(8)), 131.7 (C(2)), 133.3 (C(1)), 141.4 (C(12)), 153.5 (C(20)), 156.5 (q, *J*=36.3 Hz, C(16)), 165.1 (C(7)); *m/z* (ES^+) 460 ($[M+Na]^+$, 85%), 401 ($[M+Na+MeCN-Boc]^+$, 100%), 360 ($[M+Na-Boc]^+$, 30%); HRMS $C_{21}H_{22}N_3O_4NaF_3^+$ requires: 460.1460, found: 460.1442.

tert-butyl _N_-{2-[4-(aminomethyl)benzamido]phenyl}carbamate (70)[109]



To a suspension of **69** (1.45 g, 3.32 mmol, 1.0 eq) in methanol (13 ml) and water (20 ml) was added potassium carbonate (0.518 g, 3.75 mmol, 1.13 eq) the mixture was stirred at 70 °C for 4 h, then cooled to rt, diluted with water (50 ml) and extracted with chloroform (75 ml). The organic layer was washed with brine (75 ml), dried Na_2SO_4 and evaporated to give **70** as a cream solid (0.994 g, 2.91 mmol, 88%), mp 80-83 °C; ν_{max} (cm^{-1}) 3278 (NH), 1693 (C=O), 1653 (C=O); ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 1.53 (9H, s, (3 × C(20)H_3), 3.97 (2H, br s, NH_2), 7.10 (1H, br s, H(17)), 7.15 (1H, td, *J*=7.7, 1.5 Hz, H(5)), 7.21 (1H, td, *J*=7.7, 1.3 Hz, H(4)), 7.27 (1H, dd, *J*=7.7, 1.5 Hz, H(6)), 7.41 (2H, d, *J*=8.3 Hz, H(11 and 13)), 7.76 (1H, dd, *J*=7.7, 1.3 Hz, H(3)), 7.94 (2H, d, *J*=8.3 Hz, H(10 and 14)), 9.23 (1H, br s, H(7)); ^{13}C NMR (125 MHz, chloroform-*d*) δ ppm 28.4 (C(20)), 46.1 (C(15)), 81.3 (C(20)), 124.6 (C(3)), 125.8 (C(6)), 125.9 (C(4)), 126.0 (C(5)), 127.2 (C(11 and 13)), 127.8 (C(10 and 14)), 130.1 (C(9)), 131.0 (C(2)), 132.8 (C(1)), 147.2 (C(12)), 154.7 (C(18)), 165.6 (C(8)); *m/z* (EI^+) 341 (M^+, 23%), 241 ($[M+H\text{-}Boc]^+$, 15%); HRMS $C_{19}H_{23}N_3O_3^+$ requires: 341.1734, found: 341.1737.

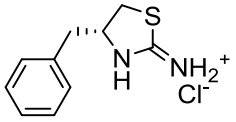
3-_tert_-butyl-1-[(2_R_)-1-hydroxy-3-phenylpropan-2-yl]thiourea (73)



The procedure by Carry *et al.* for a similar reaction was used.[110] To a solution of **72** (0.302 g, 2.0 mmol, 1.0 eq) in ethanol (3 ml) was added *tert*-butyl isothiocyanate (0.38 ml, 3.0 mmol, 1.5 eq), and then stirred at rt for 16 h. The solvent was evaporated then the colourless oil was dissolved in ethyl acetate (20 ml) and washed with brine (2 × 20 ml). Dried over Na_2SO_4 and then concentrated to give **73** as a sticky oil (0.521 g, 1.96 mmol, 98%); ν_{max} (cm^{-1}) 3414 (NH), 3286 (NH), 1532 (C=S); ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 1.34 (9H, s, C(CH_3)$_3$), 2.50 (1H, br s, OH), 2.95 (1H, dd, *J*=13.6,

8.3 Hz, C(6)H), 3.09 (1H, dd, *J*=13.6, 6.0 Hz, C(6)H`), 3.68 (1H, dd, *J*=11.0, 4.0 Hz, C(4)H), 3.77 (1H, dd, *J*=10.8, 4.0 Hz, C(4)H`), 4.70 (1H, br s, H(5)), 6.11 (1H, d, *J*=7.8 Hz, N(1)H), 6.15 (1H, br s, N(3)H), 7.18 - 7.40 (5H, m, ArH); ^{13}C NMR (75 MHz, chloroform-*d*) δ ppm 29.4 (C(*C*H_3)$_3$), 37.0 (C(6)), 52.8 (N*C*(CH$_3$)$_3$), 57.6 (C(5)), 63.2 (C(4)), 126.8 (C(10)), 128.7 (C(8 and 12)), 129.4 (C(9 and 11)), 137.6 (C(7)), 180.5 (C(2)); *m/z* (EI$^+$) 266 (M$^+$, 34%), 248 ([M-water]$^+$, 32%); HRMS $C_{14}H_{22}N_2OS^+$ requires: 266.1447, found: 266.1452.

(4*R*)-4-benzyl-1,3-thiazolidin-2-iminium chloride (74)



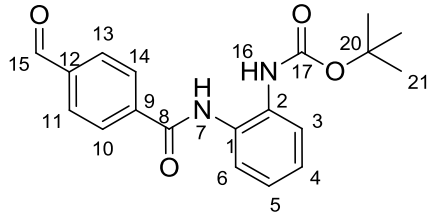
The procedure by Carry *et al.* for a similar reaction was used.[110] To **73** (0.5 g, 1.88 mmol) was added hydrochloric acid (6M, aq., 5 ml) and the resulting mixture was stirred at 100 °C under reflux for 8.5 h (the mixture became homogeneous upon heating). Then cooled to rt and stored in a refrigerator O/N (because the rotary evaporator for removing water was unavailable). The solution was evaporated to give a pale yellow oil which was triturated with diethyl ether, decanting the diethyl ether and adding fresh diethyl ether several times to give a white solid. The solid was suspended in dichloromethane (25 ml) and stirred at rt for 3 h, then filtered and washed with dichloromethane to give **74** as a white solid (0.252 g, 1.10 mmol, 59 %). Some of the free base was also liberated: a portion of the solid was suspended in dichloromethane and washed with sodium hydrogen carbonate (sat. aq.), brine and then dried with Na_2SO_4 and concentrated.

HCl salt, mp 166-167 °C; ν_{max} (cm^{-1}) 3443 (NH), 3302 (NH), 3025 (NH), 1636 (C=N); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 2.89 (1H, dd, *J*=13.7, 7.6 Hz, H(6)), 2.99 (1H, dd, *J*=13.7, 5.6 Hz, H(6)`), 3.24 (1H, dd, *J*=11.3, 5.8 Hz, H(5)), 3.52 (1H, dd, *J*=11.3, 7.6 Hz, H(5)`), 4.50 (1H, tdd, *J*=7.6, 7.6, 5.8, 5.6 Hz, H(4)), 7.22 - 7.36 (5H, m, Ph), 8.92 - 10.38 (3H, m, 3 × NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 34.4 (C(5)), 38.6 (C(6)), 61.6 (C(4)), 126.8 (C(7)), 128.6 (C(8 and 12)), 129.4 (C(9 and 11)), 136.3 (C(10)), 172.1 (C(2)); *m/z* (CI$^+$) 193 ([M-Cl]$^+$, 35%); HRMS $C_{10}H_{13}N_2S^+$ requires: 193.0799, found: 193.0806.

Free base: mp 69-70 °C; ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 2.76 (1H, dd, *J*=13.5, 7.9 Hz, H(5), 2.94 - 3.12 (2H, m, H(5)` and H(6)), 3.28 (1H, dd, *J*=10.7, 7.2 Hz, H(6)`), 4.45 (1H, app. quin, *J*=7.2 Hz, H(4)), 5.03 (2H, br s, NH_2), 7.15 - 7.35 (5H, m,

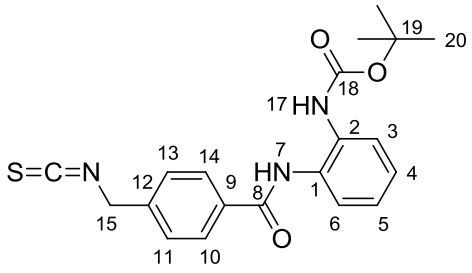
Ph); ^{13}C NMR (75 MHz, chloroform-*d*) δ ppm 39.9 (C(5)), 41.5 (C(6)), 73.8 (C(4)), 126.3 (C(7)), 128.5 (C(8 and 12)), 129.2 (C(9 and 11)), 139.2 (C(10)), 160.9 (C(2)).

tert-butyl _N_-[2-(4-formylbenzamido)phenyl]carbamate (75)[114]



To a solution of **43** (0.20 g, 0.55 mmol, 1.0 eq) in dichloromethane (3 ml) and DMSO (1 ml) at 0 °C was added trimethylamine *N*-oxide dehydrate (0.244 g, 2.2 mmol, 4.0 eq) then the resulting solution was allowed to warm to rt and stirred for 17 h. Diluted with ethyl acetate (30 ml) and washed with water (10 ml) then brine (10 ml), dried $MgSO_4$ and evaporated. Purification by column chromatography on silica gel (ethyl acetate:hexane, 1:2) gave **75** as a white solid (0.084 g, 0.247 mmol, 45%), mp 131-132 °C; ν_{max} (cm^{-1}) 3285 (NH), 1703 (C=O), 1662 (C=O); ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.50 (9H, s, H(20)), 7.02 (1H, s, H(16)), 7.06 - 7.21 (3H, m, H(4), H(5) and H(6), 7.71 - 7.80 (1H, m, H(3)), 7.95 (2H, d, *J*=8.4 Hz, H(11 and 13)), 8.10 (2H, d, *J*=8.4 Hz, H(10 and 14)), 9.62 (1H, br s, H(7)), 10.08 (1H, s, H(15)); ^{13}C NMR (75 MHz, chloroform-*d*) δ ppm 28.3 (C(20)), 81.6 (C(19)), 124.5 (C(3)), 125.8 (C(6)), 126.0 (C(4)), 126.2 (C(5)), 128.1 (C(10 and 14)), 129.8 (C(11 and 13)), 129.9 (C(2)), 130.5 (C(1)), 138.4 (C(9)), 139.5 (C(12)), 154.8 (C(17)), 164.5 (C(8)), 191.6 (C(15)); *m/z* (ES^-) 339 ($[M-H]^-$, 34%); HRMS $C_{19}H_{19}N_2O_4^-$ requires: 339.1345, found: 339.1347.

tert-butyl _N_-{2-[4-(isothiocyanatomethyl)benzamido]phenyl}carbamate (77)

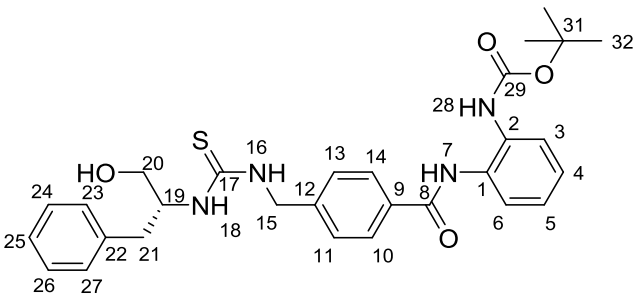


According to a literature procedure,[117] carbon disulfide (3.48 mL, 57.5 mmol, 3.2 eq) was added to a mixture of 4-(aminomethyl)benzoic acid (3.0 g, 19.8 mmol, 1.1 eq) and triethylamine (6.62 mL, 47.5 mmol, 2.6 eq) in THF (13 mL) and water (13 mL). The reaction mixture was stirred vigorously for 22 h, then the resulting solution was cooled to 0 °C and a solution of iodine (5.38 g, 21.2 mmol, 1.18 eq) in THF(13 mL)

was added dropwise over 10 min. The mixture was stirred at 0 °C for 2 h, then 1M hydrochloric acid (20 mL) and sodium sulphite (0.49 g, 3.9 mmol) were added and mixed. The mixture was then extracted with ethyl acetate (100 mL and 50 mL), the combined organics were washed with 1M hydrochloric acid (2 × 50 mL) and brine (50 mL) then dried $MgSO_4$ and evaporated to give 4-(isothiocyanatomethyl)benzoic acid as a cream

solid (4.07 g, 21.1 mmol, 106%). To a suspension of this cream solid in dichloromethane (35 mL) was added oxalyl chloride (2.28 mL, 27 mmol, 1.5 eq) dropwise, followed by a few drops of DMF. The resulting mixture was stirred at rt for 1 h (until bubble formation had ceased) and the resulting solution stirred at reflux for 1 h. The solution was evaporated and then co-evaporated with toluene (20 mL) to ensure complete removal of excess oxalyl chloride. The remaining oil was dissolved in dichloromethane (25 mL) and added dropwise over 10 min to a solution of **39** (3.748 g, 18 mmol, 1 eq) in dichloromethane (25 mL) and pyridine (25 mL) at 0 °C. The reaction was stirred at 0 °C for 1 h then warmed to rt and stirred for a further 16.5 h. Then sodium hydrogen carbonate (sat. aq., 150 mL) was added and the mixture extracted with chloroform (2 × 100 mL). The combined organics were washed with 1M hydrochloric acid (2 × 150 mL) and brine (150 mL), then dried $MgSO_4$ and evaporated to give an orange oil that was purified by column chromatography (3% ethyl acetate/dichloromethane) to give **77** as a cream solid (5.11 g, 13.3 mmol, 74%).

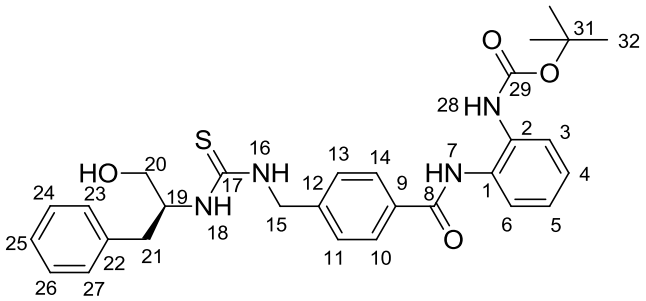
tert-butyl (*R*)-(2-(4-((3-(1-hydroxy-3-phenylpropan-2-yl)thioureido)methyl)benzamido)phenyl)carbamate ((*R*)-79a)



D-Phenylalaninol (0.126 g, 0.83 mmol) was reacted according to general procedure A and the product purified by column chromatography on silica gel (ethyl acetate:hexane, 2:1) to give **(*R*)-79a** as a white solid (0.382 g, 94%), mp 101−103 °C; ν_{max} (cm^{-1}) 3279 (NH), 1692 (C=O), 1648 (C=O); 1H NMR (400 MHz, chloroform-*d*) δ 1.49 (9H, s, H(32)), 1.88 (1H, s, H(16/18)), 2.72 (1H, dd, J = 13.6, 8.0 Hz, H(21)), 2.84 (1H, dd, J = 13.6, 5.8 Hz, H(21)'), 3.37 (2H, br s, OH and H(20)), 3.60 (1H, d, J = 10.5

Hz, H(20')), 4.28−5.00 (3H, m, H(15), H(15)' and H(19)), 6.65 (1H, br s, H(28)), 7.06−7.37 (10H, m, ArH), 7.60 (1H, d, J = 7.3 Hz, H(6)), 7.67 (2H, d, J = 7.8 Hz, H(10 and 14)), 9.38 (1H, br s, H(7)); ^{13}C NMR (150 MHz, chloroform-*d*) δ ppm 28.4 (C(32)), 37.3 (C(21)), 48.0 (C(15)), 57.2 (C(20)), 64.1 (C(19)), 81.8 (C(31)), 124.9 (C(3)), 125.9 (C(6)), 126.1 (C(4)), 126.6 (C(5)), 126.8 (C(25)), 127.5 (C(10 and 14)), 127.8 (C(11 and 13)), 128.8 (C(23 and 27)), 129.3 (C(24 and 26)), 130.4 (C(9)), 130.7 (C(2)), 132.6 (C(1)), 137.5 (C(22)), 142.5 (C(12)), 154.9 (C(28)), 166.3 (C(8)), 182.8 (C(17)); m/z (ES, %) 557 ([M+Na]$^+$, 100); HRMS (CI) calcd for $C_{29}H_{35}N_4O_4S$ [M+H]$^+$ 535.2374, found 535.2378.

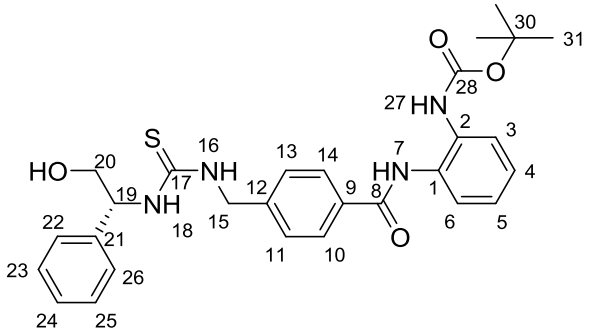
tert-butyl (*S*)-(2-(4-((3-(1-hydroxy-3-phenylpropan-2-yl)thioureido)methyl)benzamido)phenyl)carbamate ((*S*)-79a)



Using a method from.[115] To a suspension of **77** (0.240 g, 0.626 mmol, 1.0 eq) in toluene (5 ml) was added L-phenylalinol **46a** (0.095 g, 0.626 mmol, 1.0 eq) and the mixture was stirred at rt under an nitrogen balloon for 63 h. The resulting solution was evaporated and then azeotroped with dichloromethane to give a white solid, which was purified by column chromatography on silica gel (ethyl acetate:hexane, 2:1) to give **(*S*)-79a** as a white solid (0.290 g, 0.543 mmol, 87%), mp 103-106 °C; ν_{max} (cm^{-1}) 3287 (NH), 1693 (C=O), 1648 (C=O); ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 1.49 (9H, s, H(32)), 1.81 (1H, br s, H(16/18)), 2.73 (1H, dd, *J*=13.7, 7.8 Hz, H(21)), 2.85 (1H, dd, *J*=13.7, 6.0 Hz, H(21)`), 3.20 (1H, br s, OH), 3.35 - 3.45 (1H, m, H(20)), 3.62 (1H, br. d, *J*=8.3 Hz, H(20)`), 4.36 - 4.84 (3H, m, H(15), H(15)' and H(19)), 6.61 (1H, br s, H(28)), 7.06 - 7.35 (11H, m, 10 ArH and H(16/18)), 7.59 - 7.66 (1H, m, H(6)), 7.69 (2H, d, *J*=8.0 Hz, H(10 and 14)), 9.35 (1H, br s, H(7)); ^{13}C NMR (150 MHz, chloroform-*d*) δ ppm 28.4 (C(32)), 37.3 (C(21)), 48.0 (C(15)), 57.2 (C(20)), 64.2 (C(19)), 81.8 (C(31)), 124.9 (C(3)), 125.9 (C(6)), 126.1 (C(4)), 126.6 (C(5)), 126.8 (C(25)), 127.5 (C(10 and 14)), 127.8 (C(11 and 13)), 128.8 (C(23 and 27)), 129.3 (C(24 and 26)), 130.4 (C(9)), 130.7 (C(2)), 132.6 (C(1)), 137.5 (C(22)), 142.5 (C(12)), 154.9 (C(28)), 166.3 (C(8)), 182.8 (C(17)); *m/z* (ES$^+$) 557 ([M+Na]$^+$, 36%), 435 ([M+H-

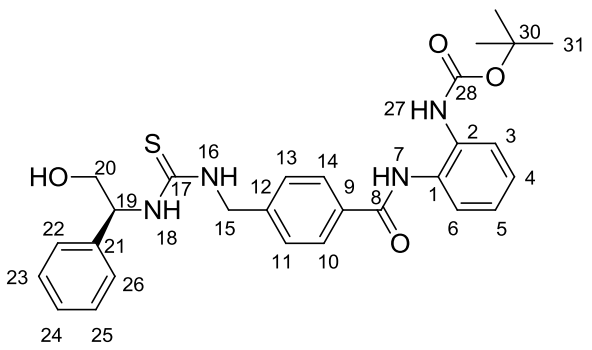
Boc]$^+$, 100%); HRMS $C_{29}H_{34}N_4O_4SNa^+$ requires: 557.2198, found: 557.2192.

***tert*-butyl (*R*)-(2-(4-((3-(2-hydroxy-1-phenylethyl)thioureido)methyl)benzamido)phenyl)carbamate ((*R*)-79b)**



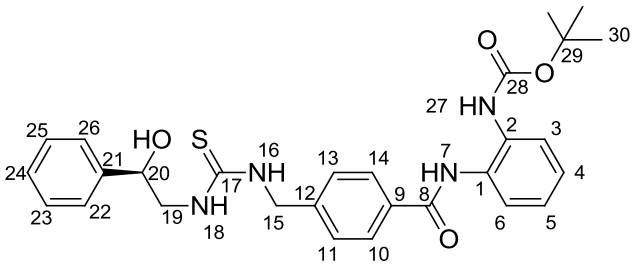
(*R*)-2-Phenylglycinol **(*R*)-46b** (0.112 g, 0.82 mmol) was reacted according to general procedure A and the product purified by column chromatography on silica gel (ethyl acetate/hexane, 3:2 to 2:1) to give **(*R*)-79b** as a white solid (0.37 g, 96%), mp 107.109 °C; ν_{max} (cm^{-1}) 3278 (NH), 1692 (C=O), 1648 (C=O); ^{1}H NMR (400 MHz, chloroform-*d*) δ 1.49 (9H, s, C(CH$_3$)$_3$), 1.88 (1H, br s, H(16/18)), 3.49 (1H, br s, OH), 3.66 (1H, m, H(20), 3.79 (1H, d, J = 8.8 Hz, H(20)'), 4.69 (2H, d, J = 2.8 Hz, H(15) and H(15)'), 5.20 (1H, br s, H(19)), 6.97.7.39 (11H, m, ArH), 7.59 (1H, d, J = 6.8 Hz, H(3)), 7.69 (2H, d, J = 8.0 Hz, H(10 and 14)), 9.35 (1H, br s, H(7)); ^{13}C NMR (150 MHz, chloroform-*d*) δ ppm 28.4 (C(31)), 48.4 (C(15)), 60.2 (C(19)), 66.5 (C(20), 81.7 (C(30)), 124.9 (C(3)), 125.9 (C(6)), 126.0 (C(4)), 126.5 (C(5)), 126.9 (C(22 and 26)), 127.4 (C(10 and 14)), 127.8 (C(11 and 13)), 128.3 (C(24)), 129.1 (C(23 and 25)), 130.4 (C(9)), 130.7 (C(2)), 132.7 (C(1)), 138.2 (C(21)), 142.3 (C(12)), 154.9 (C(28)), 166.2 (C(8)), 182.7 (C(17)); m/z (ES, %) 521 ([M+H]$^+$, 80%), 421 ([M+H-(Boc)]$^+$, 100%); HRMS calcd for $C_{28}H_{33}N_4O4_S$ [M+H]$^+$ 521.2217, found 521.2195.

***tert*-butyl (*S*)-(2-(4-((3-(2-hydroxy-1-phenylethyl)thioureido)methyl)benzamido)phenyl)carbamate ((*S*)-79b)**



To a solution of (*S*)-2-phenylglycinol, **(*S*)-46b** (0.165 g, 1.2 mmol, 1.2 eq) in acetonitrile (4 ml) was added isothiocyanate **77** (0.384 g, 1.0 mmol, 1.0 eq) and the resulting suspension stirred at rt for 16.5 h. The resulting solution was evaporated, then dissolved in ethyl acetate (20 ml) and washed with hydrochloric acid (1M aq., 20 ml), sodium hydrogen carbonate (sat. aq., 20 ml) and brine (20 ml). The organic was dried with $MgSO_4$ and evaporated then purified by column chromatography on silica gel (ethyl acetate:hexane, 3:2 to 2:1) to give **(*S*)-79b** as a white solid (0.464 g, 0.891 mmol, 89%), mp 108-111 °C; ν_{max} (cm^{-1}) 3285 (NH), 1691 (C=O), 1646 (C=O); ^{1}H NMR (600 MHz, chloroform-*d*) δ ppm 1.46 (9H, s, H(31)), 1.84 (1H, s, H(16/18), 3.45 (1H, br s, OH), 3.61 - 3.69 (1H, m, H(20)), 3.73 - 3.81 (1H, m, H(20)`), 4.53 - 4.81 (2H, m, H(15) and H(15)'), 5.19 (1H, br s, H(19)), 7.08 (2H, br s, ArH and H(27)), 7.11 - 7.18 (3H, m, ArH), 7.20 (2H, d, *J*=7.2 Hz, ArH), 7.24 - 7.32 (5H, m, 4 ArH and H(16/18)), 7.59 (1H, d, *J*=6.8 Hz, H(3)), 7.67 (2H, d, *J*=7.5 Hz, H(10 and 14)), 9.33 (1H, br s, H(7)); ^{13}C NMR (150 MHz, chloroform-*d*) δ ppm 28.4 (C(31)), 48.4 (C(15)), 60.2 (C(19)), 66.5 (C(20), 81.7 (C(30)), 124.9 (C(3)), 125.9 (C(6)), 126.0 (C(4)), 126.5 (C(5)), 126.9 (C(22 and 26)), 127.4 (C(10 and 14)), 127.8 (C(11 and 13)), 128.3 (C(24)), 129.1 (C(23 and 25)), 130.4 (C(9)), 130.7 (C(2)), 132.7 (C(1)), 138.2 (C(21)), 142.3 (C(12)), 154.9 (C(28)), 166.2 (C(8)), 182.7 (C(17)); *m/z* (ES^+) 521 ($[M+H]^+$, 20%), 543 ($[M+Na]^+$, 50%); HRMS $C_{28}H_{33}N_4O_4S^+$ requires: 521.2223, found: 521.2180.

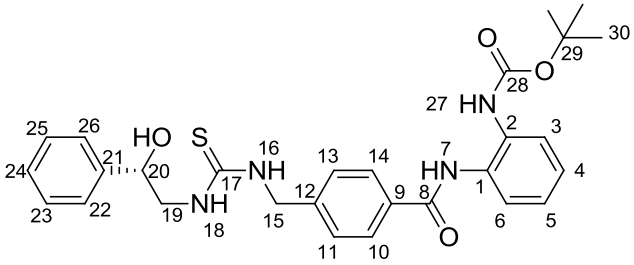
(*R*)-*tert*-butyl (2-(4-((3-(2-hydroxy-2-phenylethyl)thioureido)methyl)benzamido)phenyl)carbamate ((*R*)-79d)



(*R*)-(-)-2-amino-1-phenylethanol (0.137 g, 1.0 mmol, 1 eq) was reacted according to general procedure A to give **(*R*)-79d** as a pale yellow solid (0.519 g, 100%) which was used without purification, mp 153-156 °C; ν_{max} (cm^{-1}) 3287 (NH), 1682 (C=O), 1660 (C=O); ^{1}H NMR (600 MHz, chloroform-*d*) δ ppm 1.48 (9H, s, $C(CH_3)_3$), 3.29 - 4.08 (3H, br. m, OH and 2 × H(19)), 4.57 - 5.01 (3H, m, H(15), H(15)' and H(20)), 6.64 (1H, br s, BocNH), 7.06 (1H, br s, ArH), 7.15 - 7.23 (2H, m, ArH), 7.24 - 7.36 (7H, m, ArH), 7.66 (1H, d, *J*=7.2 Hz, H(6)), 7.73 (2H, d, *J*=7.2 Hz, H(10 and 14)),

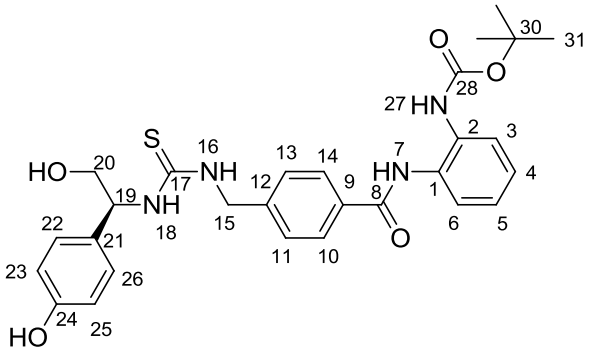
9.36 (1H, br s, H(7)); ^{13}C NMR (150 MHz, chloroform-*d*) δ ppm 28.4 (C(*C*H$_3$)$_3$), 48.5 (C(15)), 52.0 (C(19)), 74.2 (C(20)), 81.9 (O*C*(CH$_3$)$_3$), 125.0 (C(3)), 125.9 (C(22 and 26)), 126.2 (C(6) and C(4)), 126.5 (C(5)), 127.7 (C(10 and 14)), 127.9 (C(11 and 13)), 128.1 (C(24)), 128.7 (C(23 and 25)), 130.4 (C(9)), 130.7 (C(2)), 132.9 (C(1)), 141.6 (C(21)), 142.2 (C(12)), 154.9 (NCOO), 166.0 (C(8)), 183.8 (C(17)); *m/z* (EI) 519 ([M-H]$^-$, 50%); HRMS (CI) calcd. for $C_{28}H_{33}N_4O_4S$ [M+H]$^+$ 521.2217, found: 521.2195.

(*S*)-*tert*-butyl (2-(4-((3-(2-hydroxy-2-phenylethyl)thioureido)methyl)benzamido)phenyl)carbamate ((*S*)-79d)



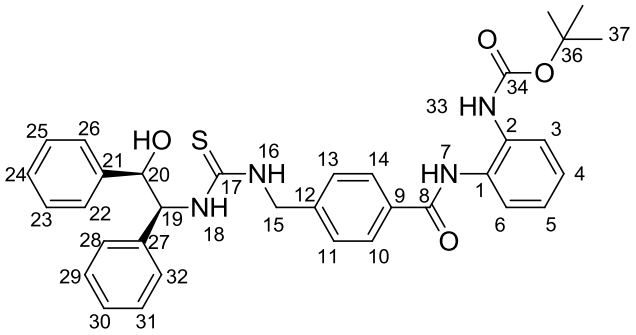
(*S*)-(+)-2-amino-1-phenylethanol (0.125 g, 0.911 mmol, 1 eq) was reacted according to general procedure A and purified by column chromatography on silica gel (ethyl acetate:hexane, 3:2), then purified again by column chromatography on silica gel (ethyl acetate:hexane, 1:1) to give **(*S*)-79d** as a white solid (0.272 g, 57%), mp 150-154 °C; ν_{max} (cm^{-1}) 3284 (NH), 1682 (C=O), 1661 (C=O); ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 1.48 (9H, s, C(CH$_3$)$_3$), 3.48 (1H, br s, OH), 3.58 - 4.35 (2H, m, 2 × H(19)), 4.69 (3H, br s, H(15), H(15)' and H(20)), 6.88 (1H, br s, BocNH), 7.10 - 7.20 (2H, m, ArH), 7.20 - 7.40 (8H, m, ArH), 7.53 - 7.62 (1H, m, ArH), 7.68 (2H, d, *J*=8.0 Hz, H(10 and 14)), 9.41 (1H, br s, H(7)); ^{13}C NMR (150 MHz, chloroform-*d*) δ ppm 28.4 (C(*C*H$_3$)$_3$), 48.3 (C(15)), 52.0 (C(19)), 74.0 (C(20)), 81.8 (O*C*(CH$_3$)$_3$), 124.9 (C(3)), 125.9 (C(22 and 26)), 126.0 (C(6)), 126.1 (C(4)), 126.6 (C(5)), 127.7 (C(10 and 14)), 127.8 (C(11 and 13)), 128.0 (C(24)), 128.6 (C(23 and 25)), 130.4 (C(9)), 130.6 (C(2)), 132.7 (C(1)), 141.6 (C(21)), 142.4 (C(12)), 154.9 (NCOO), 166.2 (C(8)), 183.7 (C(17)); *m/z* (EI) 519 ([M-H]$^-$, 35%); HRMS (CI) calcd. for $C_{28}H_{33}N_4O_4S$ [M+H]$^+$ 521.2217, found: 521.2208.

(*S*)-*tert*-butyl (2-(4-((3-(2-hydroxy-1-(4-hydroxyphenyl)ethyl)thioureido)methyl)benzamido)phenyl)carbamate (79e)



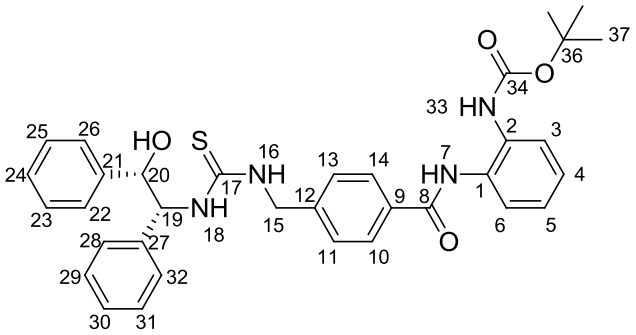
To a suspension of impure amino alcohol (**46e**) (0.536 g, 3.5 mmol) in acetonitrile (12 mL) was added **22** (0.384 g, 1.0 mmol). The resulting mixture was stirred vigorously at rt for 17.5 h before being evaporated and the residue partitioned between ethyl acetate (100 mL) and aqueous hydrochloric acid (1M, 50 mL). The organic layer was washed with water (50 mL), brine (50 mL), dried $MgSO_4$ and evaporated. Purification of the yellow solid by column chromatography on silica gel (ethyl acetate:hexane, 3:1) gave **79e** as a white solid (0.359 g, 67%), mp 128-132 °C; ν_{max} (cm^{-1}) 3250 (NH), 1685 (C=O), 1646 (C=O); ^{1}H NMR (400 MHz, methanol-d_4) δ ppm 1.51 (9H, s, $C(CH_3)_3$), 3.79 (2H, app. d, *J*=5.8 Hz, 2 × H(20)), 4.62 - 4.82 (1H, m, H(15)), 4.86 - 5.00 (1H, m, H(15)'), 5.27 (1H, br s, H(19)), 6.79 (2H, d, *J*=8.5 Hz, H(23 and 25)), 7.12 - 7.29 (4H, m, ArH), 7.30 - 7.41 (2H, m, ArH), 7.42 - 7.50 (1H, m, ArH), 7.58 - 7.66 (1H, m, ArH), 7.90 (2H, d, *J*=8.0 Hz, H(10 and 14)); ^{13}C NMR (150 MHz, methanol-d_4) δ ppm 28.7 ($C(CH_3)_3$), 60.4 (C(15)), 66.4 (C(20)), 79.5 (C(19)), 81.8 ($OC(CH_3)_3$), 116.3 (C(23 and 25)), 125.6 (C(3)), 126.3 (C(6)), 127.2 (C(4)), 127.5 (C(5)), 128.5 (C(11 and 13)), 128.7 (C(10 and 14)), 129.3 (C(22 and 26)), 131.6 (C(9) and C(2)), 133.0 (C(1)), 134.0 (C(21)), 144.9 (C(12)), 156.3 (NCOO), 158.0 (C(24)), 168.2 (C(8)), 183.9 (C(17)); *m/z* (ES) 535 ($[M-H]^-$, 100%); HRMS calcd. for $C_{28}H_{31}N_4O_5S$ $[M-H]^-$ 535.2015, found: 535.2017.

***tert*-butyl (2-(4-((3-((1*S*,2*R*)-2-hydroxy-1,2-diphenylethyl)thioureido)methyl)benzamido)phenyl)carbamate ((R,S)-79f)**



(1*R*,2*S*)-(-)-2-amino-1,2-diphenyl ethanol (0.213 g, 1.0 mmol, 1 eq) was reacted according to general procedure A to give **(R,S)-79f** as a pale yellow solid (0.596 g, 100%) which was used without purification, mp 135-140 °C; ν_{max} (cm^{-1}) 3273 (NH), 1693 (C=O), 1651 (C=O); ^{1}H NMR (600 MHz, chloroform-*d*) δ ppm 1.46 (9H, s, $C(CH_3)_3$), 3.21 (1H, br s, OH), 4.54 (1H, dd, *J*=15.1, 4.1 Hz, H(15)), 4.73 (1H, br. d, J=15.0 Hz, H(15)'), 5.06 (1H, d, *J*=3.4 Hz, H(19)), 5.51 (1H, br s, H(20)), 6.87 - 7.10 (8H, m, BocNH and ArH), 7.12 - 7.25 (8H, m, ArH), 7.58 - 7.70 (3H, m, ArH), 9.33 (1H, br s, H(7)); ^{13}C NMR (150 MHz, chloroform-*d*) δ ppm 28.4 ($C(CH_3)_3$), 48.3 (C(15)), 63.8 (C(19)), 76.6 (C(20)), 81.7 ($OC(CH_3)_3$), 124.8 (C(3)), 125.9 (C(6)), 126.0 (C(4)), 126.4 (C(5)), 126.6 (C(28 and 32)), 127.5 (C(10 and 14)), 127.8 (C(11 and 13)), 128.0 (C(24) and C(30)), 128.2 (C(22 and 26)), 128.2 (C(29 and 31)), 128.3 (C(23 and 25)), 130.3 (C(9)), 130.5 (C(2)), 133.0 (C(1)), 136.4 (C(27)), 139.6 (C(21)), 142.1 (C(12)), 154.8 (NCOO), 166.1 (C(8)), 182.4 (C(17)); *m/z* (EI) 595 ($[M-H]^-$, 60%); HRMS (CI) calcd. for $C_{34}H_{37}N_4O_4S$ $[M+H]^+$ 597.2530, found: 597.2524.

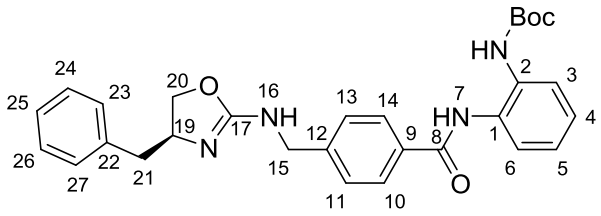
***tert*-butyl (2-(4-((3-((1*R*,2*S*)-2-hydroxy-1,2-diphenylethyl)thioureido)methyl)benzamido)phenyl)carbamate ((S,R)-79f)**



(1*S*,2*R*)-(+)-2-amino-1,2-diphenyl ethanol (0.213 g, 1.0 mmol, 1 eq) was reacted according to general procedure A to give **(S,R)-79f** as a pale yellow solid (0.590 g,

99%) which was used without purification, mp 130-133 °C; ν_{max} (cm^{-1}) 3275 (NH), 1693 (C=O), 1650 (C=O); ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 1.49 (9H, s, $C(CH_3)_3$), 3.21 (1H, br s, OH), 4.56 (1H, dd, *J*=15.4, 4.9 Hz, H(15)), 4.76 (1H, br. d, *J*=15.0 Hz, H(15)'), 5.08 (1H, d, *J*=3.8 Hz, H(19)), 5.51 (1H, br s, H(20)), 6.85 - 7.11 (8H, m, BocNH and ArH), 7.12 - 7.30 (8H, m, ArH), 7.58 - 7.74 (3H, m, ArH), 9.35 (1H, br s, H(7)); ^{13}C NMR (150 MHz, chloroform-*d*) δ ppm 28.4 ($C(CH_3)_3$), 48.3 (C(15)), 63.8 (C(19)), 76.7 (C(20)), 81.7 ($OC(CH_3)_3$), 124.8 (C(3)), 125.9 (C(6)), 126.1 (C(4)), 126.4 (C(5)), 126.6 (C(28 and 32)), 127.5 (C(10 and 14)), 127.8 (C(11 and 13)), 128.0 (C(24 and 30)), 128.2 (C(22 and 26)), 128.2 (C(29 and 31)), 128.3 (C(23 and 25)), 130.3 (C(9)), 130.5 (C(2)), 133.0 (C(1)), 136.4 (C(27)), 139.6 (C(21)), 142.1 (C(12)), 154.8 (NCOO), 166.1 (C(8)), 182.4 (C(17)); *m/z* (EI) 595 ($[M-H]^-$, 80%); HRMS (CI) calcd. for $C_{34}H_{37}N_4O_4S$ $[M+H]^+$ 597.2530, found: 597.2516.

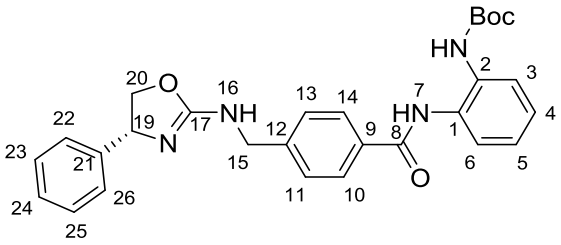
***tert*-butyl (*S*)-(2-(4-(((4-benzyl-4,5-dihydrooxazol-2-yl)amino)methyl)benzamido)phenyl)carbamate ((*S*)-80a)**



To a solution of **(*S*)-79a** (0.524 g, 0.98 mmol, 1.0 eq) in ethanol (7 mL) and toluene (14 mL) was added freshly prepared yellow mercuric oxide (0.58 g, 2.6 mmol, 2.7 eq) the resulting mixture was stirred at reflux for 30 min. Cooled to rt and filtered through Celite, evaporation of the filtrate gave **(*S*)-80a** as a white solid (0.45 g, 0.90 mmol, 92%), mp 200-201 °C; ν_{max} (cm^{-1}) 3308 (NH), 1664 (C=O), 1650 (C=O); ^{1}H NMR (500 MHz, chloroform-*d*) δ ppm 1.51 (9H, s, $C(CH_3)_3$), 2.66 (1H, dd, *J*=13.5, 8.5 Hz, H(21)), 3.06 (1H, dd, *J*=13.5, 4.7 Hz, H(21)'), 4.03 (1H, t, *J*=7.1 Hz, H(20)), 4.22 (1H, t, *J*=8.2 Hz, H(20)), 4.28 - 4.36 (1H, m, H(19), 4.43 (1H, d, *J*=15.3 Hz, H(15)), 4.47 (1H, d, *J*=15.3 Hz, H(15)'), 6.93 (1H, br s, NHBoc), 7.13 - 7.32 (5H, m, ArH), 7.38 (2H, d, *J*=7.9 Hz, H(9 and 14)), 7.79 (1H, d, *J*=7.7 Hz, H(6)), 7.92 (2H, d, *J*=7.9 Hz, H(10 and 14)), 9.19 (1H, br s, H(7)); ^{13}C NMR (125 MHz, chloroform-*d*) δ ppm 28.4 ($C(CH_3)_3$), 42.4 (C(22)), 46.7 (C(15)), 65.1 (C(19)), 72.5 (C(20)), 81.5 (C(21)), 124.6 (C(3)), 125.8 (C(6)), 126.0 (C(4)), 126.1 (C(5)), 126.5 (C(25)), 127.4 (C(11 and 13)), 127.8 (C(23 and 24)), 128.6 (C(10 and 14)), 129.3 (C(23 and 25)), 130.0 (C(9)), 131.0 (C(2)), 133.5 (C(1)), 138.0 (C(22)), 142.9 (C(12)), 154.7 (NCOO), 160.8 (C(17)),

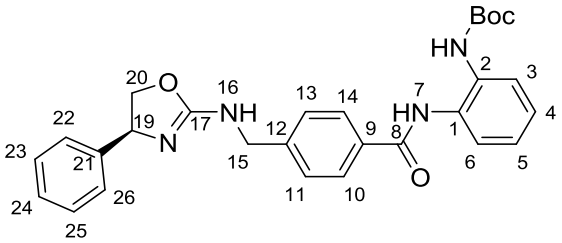
165.4 (C(8)); *m/z* (ESI$^-$) 499 ([M-H]$^-$, 100%); HRMS $C_{29}H_{31}N_4O_4^-$ requires: 499.2351, found:499.2358.

***tert*-butyl (*R*)-(2-(4-(((4-phenyl-4,5-dihydrooxazol-2-yl)amino)methyl)benzamido)phenyl)carbamate ((*R*)-80b)**



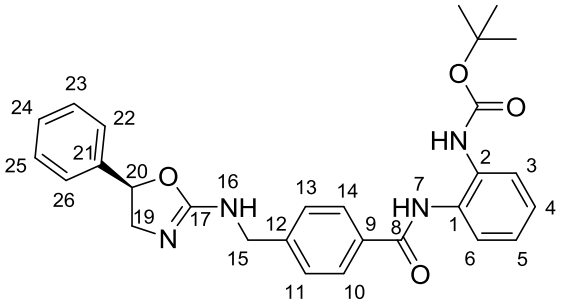
To a solution of amino alcohol **(*R*)-46b** (110 mg, 0.8 mmol) in THF (12 mL) was added isothiocyanate **77** (337 mg, 0.88 mmol), the resulting solution was stirred at rt for 18 h. The solvent was evaporated and the residue dissolved in acetone (6 mL), then iodomethane (0.1 mL, 1.6 mmol) was added and the solution stirred at rt for 9 h. Sodium bicarbonate (sat. aq., 2 mL) was added and the mixture was stirred for 10 min before being diluted with sodium bicarbonate (sat. aq., 20 mL) then extracted with ethyl acetate (2 × 20 mL). The combined organic layers were washed with brine (20 mL) dried Na_2SO_4 and evaporated to give a white solid (445 mg) that was purified by column chromatography on silica gel (5% methanol/ethyl acetate) to give **(*R*)-80b** (159 mg, 0.327 mmol, 41%) as a white solid, mp 104-106 °C; ν_{max} (cm^{-1}) 3277 (NH), 1653 (C=O); ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.48 (9H, s, C(CH_3)$_3$), 4.08 (1H, t, *J*=7.5 Hz, H(20)), 4.44 (2H, s, H(15) and H(15)'), 4.63 (1H, t, *J*=8.6 Hz, H(20)'), 5.09 (1H, dd, *J*=8.9, 7.3 Hz, H(19)), 7.03 - 7.39 (13H, m, ArH and BocNH), 7.65 - 7.74 (1H, m, ArH), 7.85 (2H, d, *J*=8.1 Hz, H(10 and 14)), 9.30 (1H, br s, H(7)); ^{13}C NMR (75 MHz, chloroform-*d*) δ ppm 28.4 (C(*C*H$_3$)$_3$), 46.7 (C(15)), 67.6 (C(19)), 75.6 (C(20)), 81.3 (O*C*(CH$_3$)$_3$), 124.7 (C(3)), 125.8 (C(6)), 125.9 (C(4)), 126.1 (C(5)), 126.6 (C(11 and 13)), 127.4 (C(22 and 26)), 127.6 (C(24)), 127.9 (C(10 and 14)), 128.8 (C(23 and 25)), 130.3 (C(9)), 130.9 (C(2)), 133.3 (C(1)), 143.1 (C(21)), 143.6 (C(12)), 154.8 (NCOO), 161.8 (C(17)), 165.7 (C(8)); *m/z* (ESI$^+$) 487 ([M+H]$^+$, 100%); HRMS $C_{28}H_{31}N_4O_4^+$ requires: 487.2340, found: 487.2336.

***tert*-butyl (*S*)-(2-(4-(((4-phenyl-4,5-dihydrooxazol-2-yl)amino)methyl)benzamido)phenyl)carbamate ((*S*)-80b)**



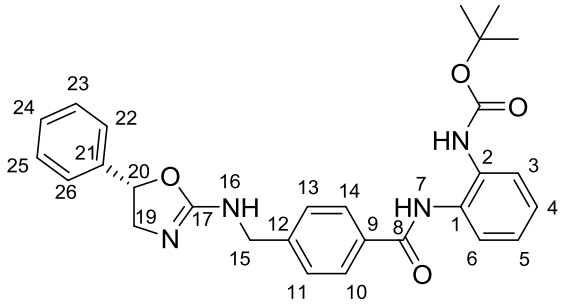
To a solution of **(*S*)-79b** (180 mg, 0.346 mmol, 1.0 eq) in ethanol (3.5 mL) and toluene (7 mL) was added yellow mercuric oxide (374 mg, 1.73 mmol, 5.0 eq) in portions. The resulting mixture was stirred at reflux for 2 hours. Filtered through Celite and the filtrate evaporated to give a white solid. This white solid was dissolved in ethanol (3.5 mL) and toluene (7 mL) then yellow mercuric oxide (374 mg, 1.73 mmol, 5.0 eq) was added and the mixture stirred at reflux for 4 h. The mixture was filtered through Celite and yellow mercuric oxide (374 mg, 1.73 mmol, 5.0 eq) was added and the mixture was stirred at reflux for 4 h. The mixture was filtered through Celite and concentrated *in vacuo* to give **(*S*)-80b** as a foamy white solid (0.127 g, 0.263 mmol, 76%), mp 100-103 °C; ν_{max} (cm^{-1}) 3274 (NH), 1654 (C=O); ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 1.51 (9H, s, $C(CH_3)_3$), 4.11 (1H, t, *J*=7.5 Hz, H(20)), 4.47 (2H, s, H(15) and H(15)'), 4.66 (1H, t, *J*=8.5 Hz, H(20)'), 5.12 (1H, dd, *J*=9.0, 7.3 Hz, H(19)), 7.15 (1H, td, *J*=7.5, 1.6 Hz, H(4)), 7.19 (1H, td, *J*=7.5, 1.6 Hz, H(5)), 7.24 - 7.30 (5H, m, ArH, and BocNH), 7.31 – 7.39 (4H, m, ArH), 7.73 (1H, d, *J*=7.3 Hz, H(6)), 7.89 (2H, d, *J*=8.0 Hz, H(10 and 14)), 9.33 (1H, br s, H(7)); ^{13}C NMR (125 MHz, chloroform-*d*) δ ppm 28.7 ($C(CH_3)_3$), 47.0 (C(15)), 67.8 (C(19)), 75.8 (C(20)), 81.6 ($OC(CH_3)_3$), 124.9 (C(3)), 126.1 (C(6)), 126.2 (C(4)), 126.3 (C(5)), 126.8 (C(11 and 13)), 127.7 (C(22 and 26)), 127.9 (C(24)), 128.1 (C(10 and 14)), 129.0 (C(23 and 25)), 130.5 (C(9)), 131.2 (C(2)), 133.6 (C(1)), 143.3 (C(21)), 143.8 (C(12)), 155.0 (NCOO), 162.0 (C(17)), 165.9 (C(8)); *m/z* (ESI$^-$) 485 ([M-H]$^-$, 95%); HRMS $C_{28}H_{29}N_4O_4^-$ requires: 485.2194, found: 485.2206.

(*R*)-*tert*-butyl (2-(4-(((5-phenyl-4,5-dihydrooxazol-2-yl)amino)methyl)benzamido)phenyl)carbamate ((*R*)-80d)



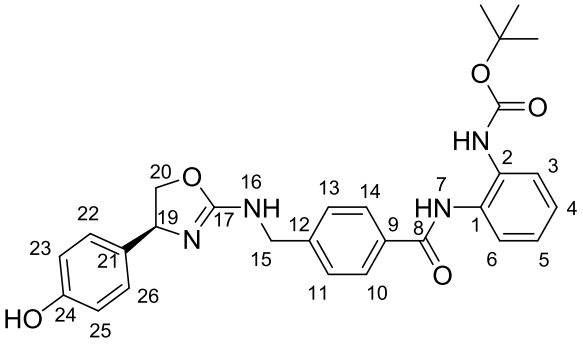
To a solution of (*R*)-2-amino-1-phenylethanol (110 mg, 0.8 mmol) in THF (8 mL) was added isothiocyanate **77** (307 mg, 0.8 mmol), the resulting solution was stirred at rt for 18 h, then evaporated. The residue was dissolved in acetone (4 mL) then iodomethane (0.100 mL, 1.600 mmol) was added and the solution stirred at rt for 6 h. Sodium bicarbonate (sat. aq., 2 mL) was added, stirred for 10 min then diluted with sodium bicarbonate (sat. aq., 20 mL), and extracted with ethyl acetate (2 × 20 mL). Combined organics were dried Na_2SO_4 and evaporated to give a yellow oil that was purified by flash column chromatography on silica gel (1-5% (2M ammonia/methanol) in dichloromethane) to give **(*R*)-80d** (291 mg, 0.598 mmol, 75%) as a white solid, mp 97-99 °C; ν_{max} (cm^{-1}) 3277 (NH), 1659 (C=O); ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 1.48 (9H, s, $C(CH_3)_3$), 3.67 (1H, dd, *J*=12.2, 7.3 Hz, H(19)), 4.14 (1H, dd, *J*=12.2, 9.2 Hz, H(19)'), 4.41 (2H, s, H(15) and H(15)'), 5.02 (1H, br s, H(16)), 5.49 (1H, t, *J*=8.3 Hz, H(20)), 7.04 - 7.19 (2H, m, BocNH and ArH), 7.22 - 7.48 (9H, m, ArH), 7.61 - 7.70 (1H, m, ArH), 7.84 (2H, d, *J*=8.3 Hz, ArH), 9.48 (1H, br s, H(7)); ^{13}C NMR (100 MHz, chloroform-*d*) δ ppm 28.3 ($C(CH_3)_3$), 46.4 (C(15)), 60.1 (C(19)), 80.9 ($OC(CH_3)_3$), 81.7 (C(20)), 124.5 (C(3)), 125.4 (C(6)), 125.6 (C(4)), 125.7 (C(11 and 13)), 125.9 (C(5)), 127.1 (C(22 and 26)), 127.8 (C(10 and 14)), 128.4 (C(24)), 128.8 (C(23 and 25)), 130.5 (C(9)), 130.6 (C(2)), 133.1 (C(1)), 140.3 (C(21)), 142.8 (C(12)), 154.7 (NCOO), 160.8 (C(17)), 165.7 (C(8)); *m/z* (ESI^+) 487 ($[M+H]^+$, 100%); HRMS $C_{28}H_{31}N_4O_4^+$ requires: 487.2340, found: 487.2342.

(*S*)-*tert*-butyl (2-(4-(((5-phenyl-4,5-dihydrooxazol-2-yl)amino)methyl)benzamido)phenyl)carbamate ((*S*)-80d)



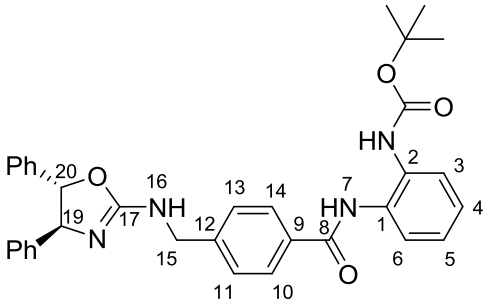
To a solution of (*S*)-2-amino-1-phenylethanol (110 mg, 0.8 mmol) in THF (8 mL) was added isothiocyanate **77** (307 mg, 0.8 mmol), the resulting solution was stirred at rt for 18 h, then evaporated. The residue was dissolved in acetone (4 mL), then iodomethane (0.10 mL, 1.6 mmol) was added and the solution stirred at rt for 4 h. Sodium bicarbonate (sat. aq., 2 mL) was added, stirred for 10 min then diluted with sodium bicarbonate (sat. aq., 20 mL), and extracted with ethyl acetate (2 × 35 mL). Combined organics were dried Na_2SO_4 and evaporated to give a white solid (396 mg) that was purified by flash column chromatography on silica gel (1-5% (2M ammonia/methanol) in dichloromethane) to give **(*S*)-80d** (275 mg, 0.565 mmol, 71%) as a white solid, mp 100-102 °C; ν_{max} (cm^{-1}) 3274 (NH), 1655 (C=O); ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 1.51 (9H, s, $C(CH_3)_3$), 3.73 (1H, dd, *J*=12.2, 7.3 Hz, H(19)), 4.20 (1H, dd, *J*=12.3, 9.2 Hz, H(19)'), 4.49 (2H, s, H(15) and H(15)'), 5.52 (1H, dd, *J*=9.2, 7.3 Hz, H(20)), 7.12 - 7.23 (2H, m, BocNH and ArH), 7.27 - 7.43 (9H, m, ArH), 7.75 (1H, d, *J*=7.6 Hz, ArH), 7.91 (2H, d, *J*=8.1 Hz, H(10 and 14)), 9.31 (1H, br s, H(7)); ^{13}C NMR (100 MHz, chloroform-*d*) δ ppm 28.3 ($C(CH_3)_3$), 46.6 (C(15)), 60.7 (C(19)), 81.2 ($OC(CH_3)_3$), 81.7 (C(20)), 124.5 (C(3)), 125.6 (C(6)), 125.7 (C(4)), 125.8 (C(11 and 13)), 125.9 (C(5)), 127.3 (C(22 and 26)), 127.8 (C(10 and 14)), 128.3 (C(24)), 128.8 (C(23 and 25)), 130.2 (C(9)), 130.8 (C(2)), 133.3 (C(1)), 140.5 (C(21)), 142.9 (C(12)), 154.6 (NCOO), 160.5 (C(17)), 165.5 (C(8)); *m/z* (ESI^+) 487 ($[M+H]^+$, 100%); HRMS $C_{28}H_{31}N_4O_4^+$ requires: 487.2340, found: 487.2327.

(*S*)-*tert*-butyl (2-(4-(((4-(4-hydroxyphenyl)-4,5-dihydrooxazol-2-yl)amino)methyl)benzamido)phenyl)carbamate (80e)



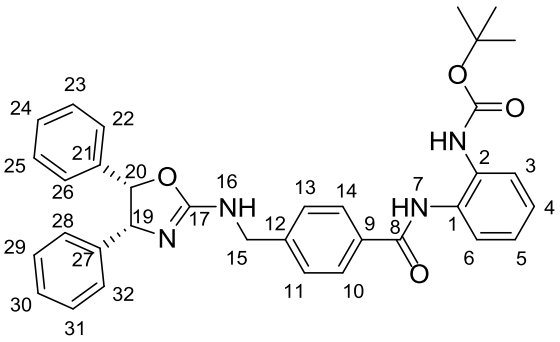
To a solution of **79e** (270 mg, 0.503 mmol) in acetone (4 mL) was added iodomethane (0.047 mL, 0.755 mmol) and the solution stirred at rt for 4 h. More iodomethane (0.047 mL, 0.755 mmol) was added and the reaction was stirred at rt for 17.5 h. Sodium bicarbonate (sat. aq., 2 mL) was added and the mixture was stirred for 10 min before being diluted with sodium bicarbonate (sat. aq., 20 mL) and water (5 mL), then extracted with ethyl acetate (2 × 30 mL). The combined organic layers were dried Na_2SO_4 and evaporated to give **80e** (252 mg, 0.501 mmol, 100%) as a white solid, mp 133-139 °C; ν_{max} (cm^{-1}) 3282 (NH), 1650 (C=O); ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 1.49 (9H, s, $C(CH_3)_3$), 4.09 (1H, t, *J*=7.5 Hz, H(20)), 4.33 - 4.50 (2H, m, H(15) and H(15)'), 4.40 - 4.55 (1H, br s, BocNH), (4.58 (1H, t, *J*=8.6 Hz, H(20)'), 4.98 (1H, dd, *J*=8.8, 7.1 Hz, H(19)), 6.58 (2H, d, *J*=8.5 Hz, H(23 and 25)), 6.91 (2H, d, *J*=8.5 Hz, H(22 and 26), 7.11 - 7.21 (2H, m, H(4) and H(5)), 7.27 - 7.35 (4H, m, H(11 and 13), H(3) and OH), 7.63 - 7.73 (1H, m, H(6)), 7.83 (2H, d, *J*=8.3 Hz, H(10 and 14)), 9.37 (1H, br s, H(7)); ^{13}C NMR (125 MHz, chloroform-*d*) δ ppm 28.3 ($C(CH_3)_3$), 46.3 (C(15)), 66.2 (C(19)), 75.7 (C(20)), 81.3 ($OC(CH_3)_3$), 115.9 (C(23 and 25)), 124.6 (C(3)), 125.6 (C(5)), 125.7 (C(6)), 126.1 (C(4)), 127.2 (C(11 and 13)), 127.5 (C(22 and 26)), 127.7 (C(10 and 14)), 130.4 (C(9)), 130.6 (C(2)), 132.9 (C(1)), 133.6 (C(21)), 142.7 (C(12)), 154.6 (NCOO), 156.3 (C(24)), 161.5 (C(17)), 165.8 (C(8)); *m/z* (ESI^+) 503 ($[M+H]^+$, 100%); HRMS $C_{28}H_{31}N_4O_5^+$ requires: 503.2289, found: 503.2281.

tert-butyl (2-(4-((((4*S*,5*S*)-4,5-diphenyl-4,5-dihydrooxazol-2-yl)amino)methyl)benzamido)phenyl)carbamate ((*R*,*R*)-80f)



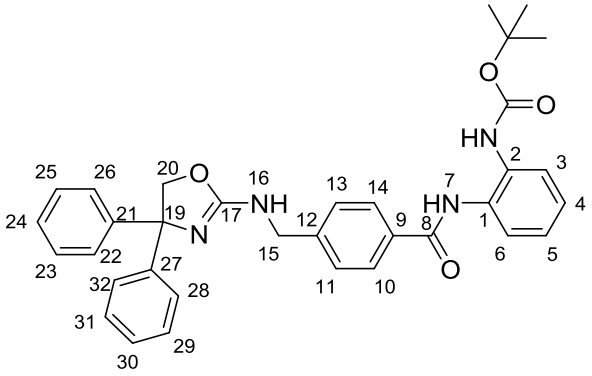
To a solution of (1*S*,2*S*)-2-amino-1,2-diphenylethanol (122 mg, 0.574 mmol) in THF (6 mL) was added isothiocyanate **77** (200 mg, 0.522 mmol), the resulting solution was stirred at rt for 16 h. The solvent was evaporated and the residue dissolved in acetone (3 mL), then iodomethane (0.098 mL, 1.565 mmol) was added and the solution stirred at rt for 5.5 h. Sodium bicarbonate (sat. aq., 1.5 mL) was added and the mixture was stirred for 10 min before being diluted with sodium bicarbonate (sat. aq., 20 mL) and water (5 mL), then extracted with ethyl acetate (2 × 30 mL). The combined organic layers were dried Na_2SO_4 and evaporated to give a white solid (260 mg) that was purified by flash column chromatography on silica gel (1-5% (2M ammonia/methanol) in dichloromethane) to give **(*R*,*R*)-80f** (165 mg, 0.293 mmol, 56%) as a white solid, mp 137-139 °C; ν_{max} (cm^{-1}) 3271 (NH), 1656 (C=O); ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 1.51 (9H, s, $C(CH_3)_3$), 4.15 (1H, br s, H(16)), 4.46 - 4.60 (2H, m, H(15) and H(15)'), 4.98 (1H, d, *J*=6.8 Hz, H(19)), 5.23 (1H, d, *J*=6.8 Hz, H(20)), 7.08 - 7.47 (16H, m, ArH and BocNH), 7.75 (1H, dd, *J*=7.8, 1.0 Hz, H(6)), 7.92 (2H, d, *J*=8.1 Hz, H(10 and 14), 9.32 (1H, br s, H(7)); ^{13}C NMR (100 MHz, chloroform-*d*) δ ppm 28.3 ($C(CH_3)_3$), 46.5 (C(15)), 76.1 (C(19)), 81.2 ($OC(CH_3)_3$), 89.6 (C(20)), 124.5 (Ar CH), 125.7 (C(6)), 125.8 (Ar 4 × CH), 125.9 (Ar CH), 126.5 (Ar 2 × CH), 127.3 (Ar 2 × CH), 127.6 (Ar CH), 127.8 (C(10 and 14)), 128.6 (Ar CH), 128.7 (Ar 2 × CH), 128.9 (Ar 2 × CH), 130.2 (quaternary), 130.8 (quaternary), 133.3 (quaternary), 139.7 (quaternary), 142.7 (quaternary), 142.9 (quaternary), 154.7 (NCOO), 160.8 (C(17)), 165.5 (C(8)); *m/z* (ESI^+) 563 ($[M+H]^+$, 100%); HRMS $C_{34}H_{35}N_4O_4^+$ requires: 563.2653, found: 563.2639.

tert-butyl (2-(4-((((4*R*,5*S*)-4,5-diphenyl-4,5-dihydrooxazol-2-yl)amino)methyl)benzamido)phenyl)carbamate ((*R*,*S*)-80f)



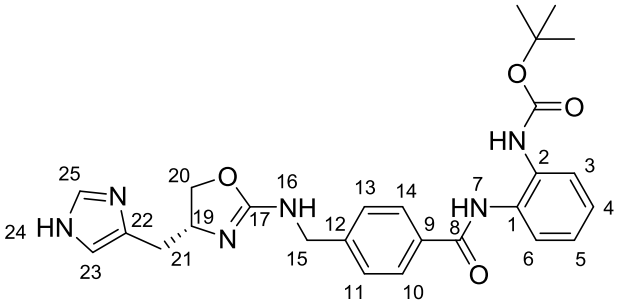
To a solution of (1*S*,2*R*)-2-amino-1,2-diphenylethanol (171 mg, 0.8 mmol) in THF (8 mL) was added isothiocyanate **77** (307 mg, 0.8 mmol), the resulting solution was stirred at rt for 18 h, then evaporated. The residue was dissolved in acetone (4 mL), then iodomethane (0.100 mL, 1.600 mmol) was added and the solution stirred at rt for 6 h. Sodium bicarbonate (sat. aq., 2 mL) was added, stirred for 10 min then diluted with sodium bicarbonate (sat. aq., 20 mL), and extracted with ethyl acetate (2 × 20 mL). Combined organics were dried Na_2SO_4 and evaporated to give a colourless oil that was purified by flash column chromatography on silica gel (1-5% (2M ammonia/methanol) in dichloromethane) then with a SCX-2 cartridge (washed with methanol and then eluted with 2M ammonia in methanol) to give **(*R*,*S*)-80f** (421 mg, 0.681 mmol, 85%) as a white solid, mp 121-123°C; ν_{max} (cm^{-1}) 3277 (NH), 1653 (C=O); ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 1.51 (9H, s, $C(CH_3)_3$), 4.48 - 4.59 (2H, m, H(15) and H(15)'), 5.40 (1H, d, *J*=8.9 Hz, H(19)), 5.84 (1H, d, *J*=8.9 Hz, H(20)), 6.84 - 6.92 (4H, m, BocNH and ArH), 6.96 - 7.09 (6H, m, ArH), 7.10 - 7.22 (2H, m, ArH), 7.28 (1H, dd, *J*=7.2, 2.1 Hz, H(6)), 7.35 - 7.46 (3H, m, ArH), 7.74 (1H, d, *J*=7.6 Hz, ArH), 7.90 (2H, d, *J*=8.1 Hz, ArH), 9.36 (1H, br s, H(7)); ^{13}C NMR (100 MHz, chloroform-*d*) δ ppm 28.3 ($C(CH_3)_3$), 46.5 (C(15)), 71.9 (C(19)), 81.1 ($OC(CH_3)_3$), 85.8 (C(20)), 124.6 (C(3)), 125.7 (C(6 and 4)), 126.0 (C(5)), 126.2 (C(10 and 14)), 126.8 (C(30)), 127.4 (C(24) and C(11 and 13)), 127.5 (C(28 and 32)), (127.6 (C(22 and 26)), 127.6 (C(29 and 31)), 127.8 (C(23 and 25)), 130.3 (C(9)), 130.7 (C(2)), 133.2 (C(1)), 136.4 (C(27)), 139.1 (C(21)), 143.1 (C(12)), 154.7 (NCOO), 161.6 (C(17)), 165.6 (C(8)); *m/z* (ESI^+) 563 ($[M+H]^+$, 100%); HRMS $C_{34}H_{35}N_4O_4^+$ requires: 563.2653, found: 563.2641.

tert-butyl (2-(4-(((4,4-diphenyl-4,5-dihydrooxazol-2-yl)amino)methyl)benzamido)phenyl)carbamate (80g)



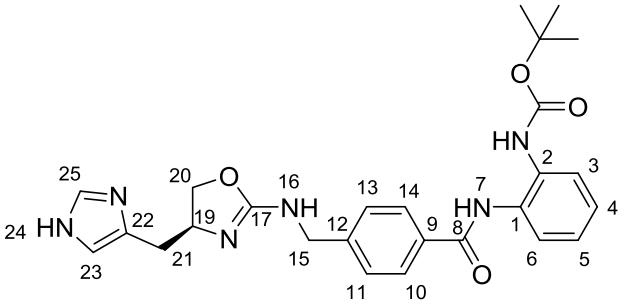
To a solution of 2-amino-2,2-diphenylethanol (**46g**) (122 mg, 0.574 mmol) in THF (6 mL) was added **77** (200 mg, 0.522 mmol), the resulting solution was stirred at rt for 24 h, then at reflux for 18 h. The solvent was evaporated and the residue dissolved in acetone (3 mL), then iodomethane (0.098 mL, 1.565 mmol) was added and the solution stirred at rt for 4 h. Sodium bicarbonate (sat. aq., 1.5 mL) was added and the mixture was stirred for 10 min before being diluted with sodium bicarbonate (sat. aq., 20 mL) and water (5 mL), then extracted with ethyl acetate (2 × 30 mL). The combined organic layers were dried Na_2SO_4 and evaporated to give a white solid (354 mg) that was purified by flash column chromatography on silica gel (1-5% (2M ammonia/methanol) in dichloromethane) to give **80g** (290 mg, 0.515 mmol, 99%) as a white solid, mp 104-106 °C; ν_{max} (cm^{-1}) 3276 (NH), 1658 (C=O); ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 1.50 (9H, s, $C(CH_3)_3$), 4.49 (2H, s, H(15) and H(15)'), 4.80 (2H, s, H(20)), 7.08 - 7.39 (16H, m, ArH and NHBoc), 7.72 (1H, d, *J*=6.8 Hz, H(6)), 7.87 (2H, d, *J*=8.3 Hz, H(10 and 14), 9.26 (1H, br s, H(7)); ^{13}C NMR (100 MHz, chloroform-*d*) δ ppm 28.3 ($C(CH_3)_3$), 46.5 (C(19)), 80.0 (C(20)), 81.2 ($OC(CH_3)_3$), 124.6 (C(3)), 125.7 (C(4)), 125.8 (C(6)), 126.0 (C(5)), 126.6 (C(22, 26, 28 and 32)), 126.9 (C(10 and 14)), 127.4 (C(11 and 13)), 127.7 (C(24 and 30)), 128.3 (C(23, 25, 29 and 31)), 130.2 (C(9)), 130.8 (C(2)), 133.1 (C(1)), 143.3 (C(12)), 146.9 (C(21 and 27)), 154.7 (NCOO), 160.1 (C(17)), 165.6 (C(8)); *m/z* (ESI^+) 563 ($[M+H]^+$, 100%); HRMS $C_{34}H_{35}N_4O_4^+$ requires: 563.2653, found: 563.2639.

(*R*)-*tert*-butyl (2-(4-(((4-((1*H*-imidazol-4-yl)methyl)-4,5-dihydrooxazol-2-yl)amino)methyl)benzamido)phenyl)carbamate ((*R*)-80h)



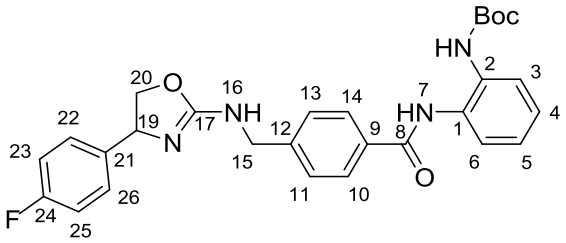
To a mixture of (*R*)-2-amino-3-(1*H*-imidazol-4-yl)propan-1-ol dihydrochloride (123 mg, 0.574 mmol) in THF (10 mL) was added potassium carbonate (159 mg, 1.147 mmol) and **77** (200 mg, 0.522 mmol) the mixture was stirred at 72 °C for 72 h. Potassium carbonate was filtered, the filtrate was evaporated and the residue dissolved in acetone (6 mL), then iodomethane (0.065 mL, 1.043 mmol) was added and the solution stirred at rt for 18 h. Sodium bicarbonate (sat. aq., 2 mL) was added and the mixture was stirred for 10 min before being diluted with sodium bicarbonate (sat. aq., 20 mL) and water (5 mL), then extracted with ethyl acetate (2 × 20 mL). The combined organic layers were washed with brine (20 mL), dried Na_2SO_4 and evaporated to give a yellow solid (250 mg), which was purified by flash column chromatography on silica gel (2 to 20% (2M ammonia/methanol) in dichloromethane) to give **(*R*)-80h** (110 mg, 0.224 mmol, 43%) as a white solid, mp 159-163 °C; ν_{max} (cm^{-1}) 3664 (NH), 3382 (NH), 1679 (C=O), 1641 (C=O); ^{1}H NMR (500 MHz, DMSO-d_6) d ppm 1.46 (9H, s, $C(CH_3)_3$), 2.62 (1H, dd, *J*=15.4, 5.8 Hz, H(21)), 3.09 (1H, dd, *J*=15.4, 7.4 Hz, H(21)'), 3.75 (1H, dd, *J*=11.0, 5.5 Hz, H(20)), 4.24 (1H, dd, *J*=11.0, 7.0 Hz, H(20)), 4.31 (2H, d, *J*=6.0 Hz, H(15) and H(15)'), 4.73 - 4.87 (1H, m, H(19)), 6.53 (1H, t, *J*=6.0 Hz, H(16)), 6.62 (1H, s, H(23)), 6.66 (1H, d, *J*=7.4 Hz, H(24)), 7.12 - 7.26 (2H, m, H(4) and H(5)), 7.41 (2H, d, *J*=8.2 Hz, H(11 and 13), 7.49 - 7.61 (3H, m, H(3), H(25) and H(6)), 7.91 (2H, d, *J*=8.0 Hz, H(10 and 14)), 8.69 (1H, br s, BocNH), 9.81 (1H, s, N(7)); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 28.5 ($C(CH_3)_3$), 29.6 (C(21)), 43.1 (C(15)), 50.7 (C(19)), 55.0 (C(20)), 80.1 ($OC(CH_3)_3$), 119.8 (C(23)), 124.4 (C(3)), 124.6 (C(4)), 126.0 (C(5)), 126.4 (C(6)), 127.4 (C(11 and 13)), 128.0 (C(10 and 14)), 130.3 (C(1)), 131.4 (C(25)), 132.1 (C(2)), 133.0 (C(9)), 134.7 (C(22)), 145.5 (C(12)), 153.9 (NCOO), 157.9 (C(17)), 165.6 (C(8)); *m/z* (ESI^+) 491 ($[M+H]^+$, 100%); HRMS $C_{26}H_{31}N_6O_4^+$ requires: 491.2401, found: 491.2390.

(*S*)-*tert*-butyl (2-(4-(((4-((1*H*-imidazol-4-yl)methyl)-4,5-dihydrooxazol-2-yl)amino)methyl)benzamido)phenyl)carbamate ((*S*)-80h)



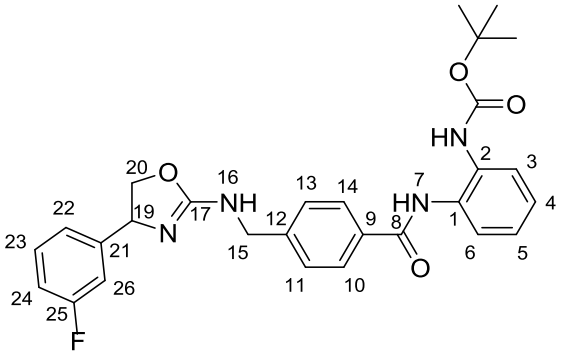
To a mixture of (*S*)-2-amino-3-(1*H*-imidazol-4-yl)propan-1-ol dihydrochloride (178 mg, 0.833 mmol) in THF (6 mL) was added potassium carbonate (230 mg, 1.666 mmol) followed by a solution of **77** (319 mg, 0.833 mmol) in THF (6 mL). The resulting mixture was stirred at rt for 17 h then reflux for 72 h. Potassium carbonate was filtered, solvents were evaporated and the residue dissolved in acetone (6 mL), then iodomethane (0.104 mL, 1.666 mmol) was added and the solution stirred at rt for 5 h. Sodium bicarbonate (sat. aq., 2 mL) was added, stirred for 10 min then diluted with sodium bicarbonate (sat. aq., 20 mL), and extracted with ethyl acetate (2 × 20 mL). Combined organics were washed with brine (20 mL), dried Na_2SO_4 and evaporated to give 389 mg of a glassy solid, which was purified by flash column chromatography on silica gel (2 to 20% (2M ammonia/methanol) in dichloromethane) to give **(*S*)-80h** (135 mg, 0.275 mmol, 33%) as a white solid, mp 155-157 ºC; ν_{max} (cm^{-1}) 3310 (NH), 1635 (C=O); ^{1}H NMR (400 MHz, methanol-*d*$_4$) δ ppm 1.51 (9H, s, $C(CH_3)_3$), 2.74 (1H, ddd, *J*=15.6, 5.5, 1.0 Hz, H(21)), 3.23 (1H, ddd, *J*=15.6, 7.3, 1.0 Hz, H(21)'), 3.87 (1H, dd, *J*=11.4, 5.3 Hz, H(20)), 4.35 (1H, dd, *J*=11.4, 6.7 Hz, H(20)'), 4.43 (2H, s, H(15) and H(15)'), 4.89 - 4.98 (1H, m, H(19)), 6.69 (1H, s, H(23)), 7.19 - 7.29 (2H, m, H(4) and H(5)), 7.40 - 7.49 (3H, m, H(11 and 13) and H(3)), 7.57 (1H, s, H(25)), 7.60 - 7.65 (1H, m, H(6)), 7.94 (2H, d, *J*=8.1 Hz, H(10 and 14)); ^{13}C NMR (100 MHz, methanol-*d*$_4$) δ ppm 27.2 ($C(CH_3)_3$), 28.8 (C(21)), 42.9 (C(15)), 50.6 (C(19)), 54.8 (C(20)), 80.3 ($OC(CH_3)_3$), 118.8 (C(23)), 124.3 (C(6)), 124.9 (C(4)), 125.7 (C(5)), 126.0 (C(2)), 126.9 (C(11 and 13)), 127.4 (C(10 and 14)), 130.3 (C(2)), 130.9 (C(25)), 131.6 (C(22)), 132.7 (C(1)), 134.6 (C(9)), 144.6 (C(12)), 154.9 (NCOO), 158.8 (C(17)), 166.7 (C(8)); *m/z* (ESI^+) 491 ($[M+H]^+$, 100%); HRMS $C_{26}H_{31}N_6O_4^+$ requires: 491.2401, found: 491.2382.

***tert*-butyl (2-(4-(((4-(4-fluorophenyl)-4,5-dihydrooxazol-2-yl)amino)methyl)benzamido)phenyl)carbamate (80i)**



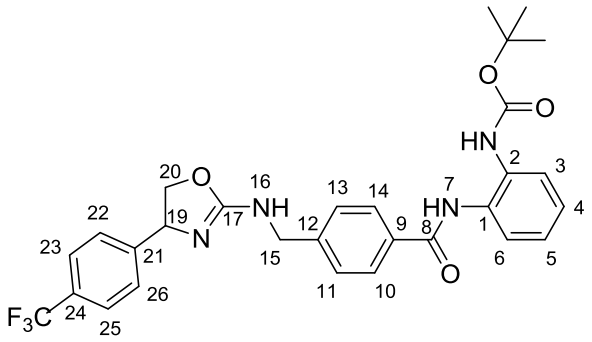
To a solution of amino alcohol **46i** (220 mg, 1.42 mmol) in THF (10 mL) was added isothiocyanate **77** (272 mg, 0.71 mmol), the resulting solution was stirred at rt for 18 h. The solvent was evaporated and the residue dissolved in acetone (5 mL), then iodomethane (0.088 mL, 1.42 mmol) was added and the solution stirred at rt for 16 h. Sodium bicarbonate (sat. aq., 2 mL) was added and the mixture was stirred for 10 min before being diluted with sodium bicarbonate (sat. aq., 20 mL) then extracted with ethyl acetate (2 × 20 mL). The combined organic layers were washed with brine (20 mL) dried Na_2SO_4 and evaporated to give a white solid (500 mg) that was purified by column chromatography on silica gel (ethyl acetate:hexane, 4:1) to give **80i** (238 mg, 0.472 mmol, 67%) as a white solid, mp 105-108 °C; ν_{max} (cm^{-1}) 3258 (NH), 1650 (C=O); ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 1.51 (9H, s, $C(CH_3)_3$), 4.06 (1H, t, *J*=7.7 Hz, H(20)), 4.50 (2H, s, H(15) and H(15)'), 4.65 (1H, t, *J*=8.5 Hz, H(20)'), 5.11 (1H, dd, *J*=8.9, 7.4 Hz, H(19)), 7.02 (2H, t, *J*=8.7 Hz, ArH), 7.12 - 7.30 (6H, m, ArH), 7.39 (2H, d, *J*=8.0 Hz, H(11 and 13)), 7.77 (1H, d, *J*=7.5 Hz, H(6)), 7.91 (2H, d, *J*=8.0 Hz, H(10 and 14)), 9.32 (1H, br s, H(7)); ^{13}C NMR (125 MHz, chloroform-*d*) δ ppm 28.3 ($C(CH_3)_3$), 46.6 (C(15)), 66.8 (C(19)), 75.4 (C(20)), 81.3 ($OC(CH_3)_3$), 115.4 (d, *J*=22.1 Hz, H(23 and 25)), 124.5 (C(3)), 125.7 (C(6)), 125.9 (C(4) and C(5)), 127.3 (C(11 and 13)), 127.7 (C(10 and 14)), 128.0 (d, *J*=8.6 Hz, C(22 and 26), 130.0 (C(9)), 130.8 (C(2)), 133.3 (C(1)), 139.2 (C(21)), 142.7 (C(12)), 154.6 (NCOO), 162.1 (d, *J*=244.7 Hz, C(24)), 161.5 (C(17)), 165.3 (C(8)); *m/z* (CI) 505 ($[M+H]^+$, 93%); HRMS $C_{28}H_{30}FN_4O_4^+$ requires: 505.2246, found: 505.2254.

tert-butyl (2-(4-(((4-(3-fluorophenyl)-4,5-dihydrooxazol-2-yl)amino)methyl)benzamido)phenyl)carbamate (80j)



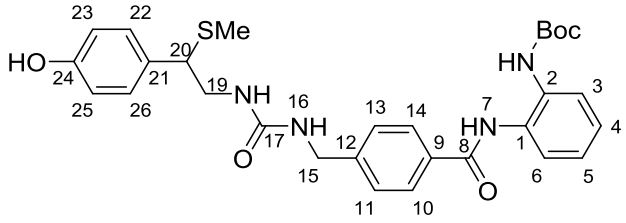
To a solution of **46j** (81 mg, 0.522 mmol) in THF (6 mL) was added isothiocyanate **77** (200 mg, 0.522 mmol), the resulting solution was stirred at rt for 16 h. The solvent was evaporated and the residue dissolved in acetone (3 mL), then iodomethane (0.098 mL, 1.565 mmol) was added and the solution stirred at rt for 4 h. Sodium bicarbonate (sat. aq., 1.5 mL) was added and the mixture was stirred for 10 min before being diluted with sodium bicarbonate (sat. aq., 20 mL) and water (5 mL), then extracted with ethyl acetate (2 × 30 mL), the combined organic layers were washed with brine, dried Na_2SO_4 and evaporated to give a white solid (286 mg). Purification by flash column chromatography on silica gel (75-100% ethyl acetate/cyclohexane) gave **80j** (200 mg, 0.396 mmol, 76%) as a white solid, mp104-107 °C; ν_{max} (cm^{-1}) 3283 (NH), 1652 (C=O); ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 1.51 (1H, s, $C(CH_3)_3$), 3.95 (1H, br s, H(16)), 4.07 (1H, dd, *J*=8.1, 7.1 Hz, H(20)), 4.44 - 4.54 (2H, m, H(15) and H(15)'), 4.64 (1H, dd, *J*=9.0, 8.1 Hz, H(20)'), 5.11 (1H, dd, *J*=9.0, 7.1 Hz, H(19)), 6.91 - 7.04 (3H, m, H(24), H(26) and BocNH), 7.11 - 7.21 (2H, m, H(4) and H(5)), 7.22 - 7.32 (3H, m, H(3), H(22) and H(23), 7.36 (2H, d, *J*=8.3 Hz, H(11 and 13)), 7.73 (1H, dd, *J*=7.8, 1.2 Hz, H(6)), 7.89 (2H, d, *J*=8.3 Hz, H(10 and 14)), 9.28 (1H, br s, H(7)); ^{13}C NMR (100 MHz, chloroform-*d*) δ ppm 28.3 ($C(CH_3)_3$), 46.6 (C(15)), 67.0 (C(19)), 75.2 (C(20)), 81.2 ($OC(CH_3)_3$), 113.3 (d, *J*=22.0 Hz, C(24)), 114.3 (d, *J*=21.3 Hz, C(26)), 122.0 (d, *J*=2.2 Hz, C(22)), 124.5 (C(3)), 125.7 (C(5)), 125.8 (C(6)), 125.9 (C(4)), 127.3 (C(11 and 13)), 127.8 (C(10 and 14)), 130.1 (d, *J*=8.1 Hz, C(23)), 130.2 (C(9)), 130.8 (C(2)), 133.3 (C(1)), 142.7 (C(12)), 146.3 (d, *J*=6.6 Hz, C(21)), 154.6 (NCOO), 161.8 (C(17)), 163.1 (d, *J*=245.8 Hz, C(25)), 165.5 (C(8)); *m/z* (ESI^+) 505 ($[M+H]^+$, 100%); HRMS $C_{28}H_{30}FN_4O_4^+$ requires: 505.2246, found: 505.2240.

tert-butyl (2-(4-(((4-(4-(trifluoromethyl)phenyl)-4,5-dihydrooxazol-2-yl)amino)methyl)benzamido)phenyl)carbamate (80k)



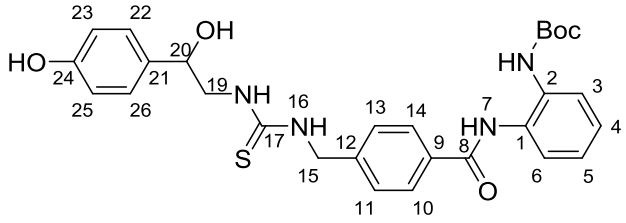
To a solution of **46k** (107 mg, 0.522 mmol) in THF (6 mL) was added **77** (200 mg, 0.522 mmol), the resulting solution was stirred at rt for 17.5 h, then evaporated. The residue was dissolved in acetone (3 mL), and iodomethane (0.065 mL, 1.043 mmol) was added the solution was then stirred at rt for 6 h, then a further 65 μL of iodomethane was added and stirred for another 2.5 h at rt. Sodium bicarbonate (sat. aq., 1.5 mL) was added, stirred for 10 min then diluted with sodium bicarbonate (sat. aq., 15 mL), and extracted with ethyl acetate (2 × 30 mL). Combined organics were dried Na_2SO_4 and evaporated to give a white solid (300 mg) that was purified by flash column chromatography on silica gel (1-5% (2M ammonia/methanol) in dichloromethane) to give **80k** (200 mg, 0.361 mmol, 69%) as a white solid, mp 114-116 °C; ν_{max} (cm^{-1}) 3282 (NH), 1658 (C=O); ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 1.49 (9H, s, $C(CH_3)_3$), 4.04 (1H, t, *J*=7.6 Hz, H(20), 4.44 (2H, s, H(15) and H(15)'), 4.65 (1H, t, *J*=8.7 Hz, H(20)'), 4.94 (1H, br s, H(16)), 5.14 (1H, t, *J*=8.1 Hz, H(19)), 7.05 - 7.18 (2H, m, ArH), 7.24 (1H, dd, *J*=7.6, 1.2 Hz, ArH), 7.31 (2H, d, *J*=8.1 Hz, ArH), 7.36 (2H, d, *J*=8.1 Hz, ArH), 7.47 (1H, br s, BocNH), 7.57 (2H, d, *J*=8.1 Hz, ArH), 7.70 (1H, d, *J*=7.1 Hz, H(6)), 7.85 (2H, d, *J*=8.1 Hz, H(10and 14)), 9.39 (1H, br s, H(7)); ^{13}C NMR (100 MHz, chloroform-*d*) δ ppm 28.3 ($C(CH_3)_3$), 46.5 (C(15)), 67.0 (C(19)), 75.0 (C(20)), 81.1 ($OC(CH_3)_3$), 124.1 (q, *J*=272.9 Hz, CF_3), 124.5 (C(3)), 125.5 (q, *J*=3.7 Hz, C(23 and 25), 125.6 (C(6 and C(4)), 125.9 (C(5)), 126.8 (C(22 and 26)), 127.2 (C(11 and 13)), 127.7 (C(10 and 14)), 129.6 (q, *J*=32.3 Hz, C(24)), 130.3 (C(9)), 130.7 (C(2)), 133.2 (C(1)), 142.8 (C(12)), 147.6 (C(21)), 154.7 (NCOO), 162.1 (C(17)), 165.6 (C(8)); *m/z* (ESI^+) 387 ($[M+H]^+$, 100%); HRMS $C_{29}H_{30}N_4O_4F_3^+$ requires: 555.2214, found: 555.2214.

tert-butyl (2-(4-((3-(2-(4-hydroxyphenyl)-2-(methylthio)ethyl)ureido)methyl)benzamido)phenyl)carbamate (81)



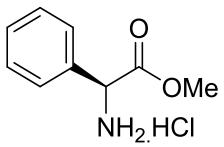
To a mixture of octopamine hydrochloride (159 mg, 0.84 mmol) and potassium carbonate (145 mg, 1.05 mmol) in THF (10 mL) was added isothiocyanate **77** (272 mg, 0.71 mmol), the resulting mixture was stirred at reflux for 90 h. The solvent was evaporated and the residue partitioned between ethyl acetate (25 mL) and water (25 mL), the layers were separated and the aqueous layer was extracted with ethyl acetate (25 mL). The combined organic layers were washed with brine (20 mL), dried Na_2SO_4 and evaporated. The residue was dissolved in acetone (5 mL), iodomethane (0.087 mL, 1.4 mmol) was added and the solution was stirred at rt for 16 h, then iodomethane (0.087 mL, 1.4 mmol) was added and stirred at rt for 24 h. Sodium bicarbonate (sat. aq., 2 mL) was added and the mixture was stirred for 10 min before being diluted with sodium bicarbonate (sat. aq., 10 mL) then extracted with ethyl acetate (2 × 20 mL). The combined organic layers were washed with brine (20 mL) dried Na_2SO_4 and evaporated to give a cream solid (400 mg) that was purified by column chromatography on silica gel (5% methanol/ethyl aceate) and again (ethyl acetate:hexane, 3:1) to give **81** (116 mg, 0.21 mmol, 30%) as a white solid, mp 125-128 °C; ν_{max} (cm^{-1}) 3285 (NH), 1640 (C=O); ^{1}H NMR (500 MHz, methanol-*d*$_4$) δ ppm 1.48 (9H, s, $C(CH_3)_3$), 1.92 (3H, s, SCH_3), 3.43 (1H, dd, *J*=13.8, 8.3 Hz, H(19)), 3.61 (1H, dd, *J*=13.8, 6.8 Hz, H(19)'), 3.84 (1H, dd, *J*=8.3, 6.8 Hz, H(20)), 4.35 (2H, s, H(15) and H(15)'), 6.72 - 6.78 (2H, m, ArH), 7.12 - 7.16 (2H, m, ArH), 7.18 - 7.26 (2H, m, ArH), 7.35 (2H, d, *J*=8.4 Hz, ArH), 7.43 (1H, dd, *J*=7.3, 2.3 Hz, ArH), 7.59 (1H, dd, *J*=7.3, 1.8 Hz, ArH), 7.86 - 7.94 (3H, m, ArH); ^{13}C NMR (125 MHz, methanol-*d*$_4$) δ ppm 14.3 (CH_3S), 28.8 ($C(CH_3)_3$), 44.4 (C(15)), 45.9 (C(19)), 52.2 (C(20)), 81.9 ($OC(CH_3)_3$), 116.5 (C(23 and 25)), 125.7 (C(3)), 126.4 (C(6)), 127.3 (C(4)), 127.6 (C(5)), 128.3 (C(11 and 13)), 128.9 (C(10 and 14)), 130.5 (C(22 and 26)), 131.8 (C(9)), 132.3 (C(2)), 133.2 (C(1)), 134.1 (C(21)), 146.3 (C(12)), 156.4 (NCOO), 158.0 (C(24)), 160.8 (C(17)), 168.4 (C(8)); *m/z* (ESI^+) 573 ($[M+Na]^+$, 28%), 403 ($[M-SMe-Boc]^+$, 100%); HRMS $C_{29}H_{34}N_4NaO_5S^+$ requires: 573.2142, found: 573.2103.

***tert*-butyl (2-(4-((3-(2-hydroxy-2-(4-hydroxyphenyl)ethyl)thioureido)methyl)benzamido)phenyl)carbamate (82)**



To a mixture of octopamine hydrochloride (159 mg, 0.84 mmol) and potassium carbonate (145 mg, 1.05 mmol) in THF (10 mL) was added isothiocyanate **77** (268 mg, 0.7 mmol), the resulting mixture was stirred at reflux for 96 h. The solvent was evaporated and the residue partitioned between ethyl acetate (25 mL) and water (25 mL), the layers were separated and the aqueous layer was extracted with ethyl acetate (10 mL). The aqueous layer was neutralised to pH 7 then extracted with ethyl acetate (20 mL). The combined organic layers were washed with brine (20 mL), dried $MgSO_4$ and evaporated to give a white solid (430 mg). Purification by column chromatography on silica gel (ethyl acetate:hexane, 2:1) gave **82** as a white solid (351 mg, 0.655 mmol, 94%), mp 134-139 °C; ν_{max} (cm^{-1}) 3276 (NH), 1692 (C=O), 1650 (C=O); ^{1}H NMR (500 MHz, methanol-d_4) δ ppm 1.48 (9H, s, $C(CH_3)_3$), 3.35 (2H, s, H(15) and H(15)'), 3.61 (1H, br s, H(19))), 3.83 (1H, br s, H(19)'), 4.60 (1H, s, OH), 4.81 (1H, br s, H(20)), 6.78 (2H, d, *J*=8.5 Hz, ArH), 7.15 - 7.27 (4H, m, ArH), 7.36 - 7.48 (3H, m, ArH), 7.60 (1H, d, *J*=7.3 Hz, ArH), 7.91 (2H, d, *J*=8.2 Hz, ArH); ^{13}C NMR (125 MHz, methanol-d_4) δ ppm 28.7 ($C(CH_3)_3$), 49.9 (C(15)), 52.7 (C(19)), 73.3 (C(20)), 81.9 ($OC(CH_3)_3$), 116.2 (C(23 and 25)), 125.6 (C(3)), 126.4 (C(6)), 127.2 (C(4)), 127.5 (C(5)), 128.5 (C(11 and 13)), 128.6 (C(10 and 14)), 128.8 (C(22 and 26)), 131.6 (C(9)), 133.0 (C(2)), 134.1 (C(1)), 134.6 (C(21)), 144.8 (C(12)), 156.3 (NCOO), 158.0 (C(24)), 168.3 (C(8)), 184.5 (br., C(17)); *m/z* (ESI^+) 537 ($[M+H]^+$, 67%), 519 ($[M+H-H_2O]^+$, 100%); HRMS $C_{28}H_{33}N_4O_5S^+$ requires: 537.2166, found: 537.2191.

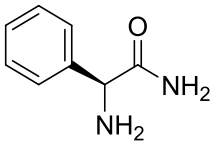
methyl (*S*)-2-amino-2-phenylacetate hydrochloride (84)[159]



To a suspension of (*S*)-phenylglycine (2.5 g, 16.5 mmol, 1.0 eq) in methanol (25 mL) was added thionyl chloride (1.3 mL, 18 mmol, 1.09 eq) dropwise, then stirred at rt

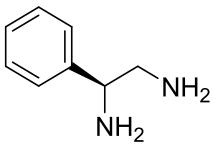
for 16 h and evaporated to give **84** as a white solid (3.33 g, 16.5 mmol, 100%), mp 189-191 °C (lit.[159] 193-195 °C).

(*S*)-2-amino-2-phenylacetamide (85)



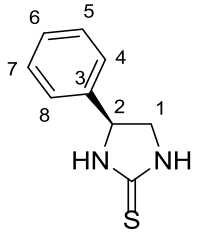
A solution of **84** (3.33 g, 16.5 mmol) in concentrated aqueous ammonia (37%, 12 mL) was stirred at rt for 16 h. The mixture was diluted with water (10 mL) and extracted with ethyl acetate (4 × 40 mL), the combined organics were dried Na_2SO_4, and concentrated to give **85** as a white crystalline solid (1.43 g, 9.5 mmol, 58%), mp 134-135 °C (lit.[160] 138-140 °C); ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 1.87 (3H, s, NH_2), 4.54 (1H, s, PhCH), 6.04 (1H, br s, N*H*H'), 6.92 (1H, br s, NH*H'*), 7.30 - 7.46 (5H, m, ArH); ^{13}C NMR (125 MHz, chloroform-*d*) δ ppm 60.3 (CHPh), 127.3 (*m*-Ph), 128.5 (*p*-Ph), 129.3 (*o*-Ph), 141.3 (*i*-Ph), 176.3 (C=O).

(*S*)-1-phenylethane-1,2-diamine (86)



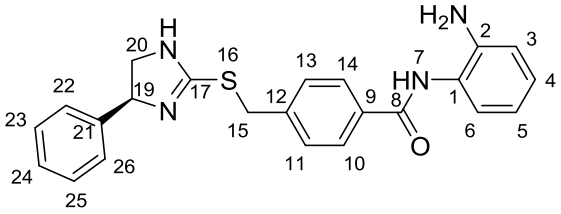
A 2-neck flask (oven-dried) was fitted with a magnetic stirrer and a reflux condenser and then $NaBH_4$ (0.703 g, 18.6 mmol, 2.0 eq) and anhydrous THF (20 ml) were added. Then a dropping funnel was fitted and the mixture cooled to 0 °C in an ice bath. A solution of iodine (3.29 g, 9.32 mmol, 1.0 eq) in anhydrous THF (10 ml) was added dropwise over 30 min, vigorous gas evolution was observed. Then acetamide **85** (1.4 g, 9.32 mmol, 1.0 eq) was added in one portion and the mixture was heated to reflux for 18 h, then cooled to rt and methanol added slowly until solution became clear. Stirred for 30 min and then the solvent was evaporated. The resulting white paste was dissolved in 20 ml of 20% aq. potassium hydroxide and stirred for 4 h then extracted with dichloromethane (3 × 30 ml). The combined organics were washed with brine, and dried Na_2SO_4 and evaporated to give an orange oil which crystallised upon standing for16 h. Trituration with a mixture of dichloromethane and hexane gave **86** as a cream solid which was still impure but used without further purification (0.5 g, 3.67 mmol, 39%), mp 178-180 °C (lit.[161] 86-89 °C [for racemate])

(*S*)-4-phenylimidazolidine-2-thione (87)



To a suspension of **86** (200 mg, 1.47 mmol) in ethanol (8 mL) and water (4 mL) was added triethylamine (0.41 mL, 2.94 mmol). To the resulting suspension at 0 °C was added dropwise carbon disulfide (0.35 mL, 5.87 mmol), the mixture was then stirred at 60 °C for 5 h. Cooled to rt then hydrochloric acid (37% aq, 0.2 mL) was added and the solution stirred at reflux for 14 h. The ethanol was evaporated and the mixture was diluted with potassium hydrogen sulfate (1M, 30 mL) and then extracted with dichloromethane (3 × 30 mL). The combined organic layers were dried with Na_2SO_4 and evaporated to give a yellow solid that was purified by column chromatography on silica gel (ethyl acetate:hexane 1:1 to 3:2) to give **87** (82 mg, 0.457 mmol, 31%) as a white solid, mp 159-160 °C (lit.[162] 184 °C [for racemate]); ν_{max} (cm^{-1}) 3217 (NH); ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 3.61 (1H, dd, *J*=9.3, 8.0 Hz, H(1)), 4.13 (1H, t, *J*=9.9 Hz, H(1)'), 5.10 (1H, t, *J*=8.8 Hz, H(2)), 6.70 (2H, br s, 2 × NH), 7.31 – 7.47 (5H, m, ArH); ^{13}C NMR (100 MHz, chloroform-*d*) δ ppm 53.9 (C(1)), 61.4 (C(2)), 126.6 (C(4 and 8)), 129.1 (C(6)), 129.5 (C(5 and 7)), 140.3 (C(3)), 184.4 (C=S); *m/z* (EI^+) 178 (M^+, 73%); HRMS $C_9H_{10}N_2S^+$ requires: 178.0565, found: 178.0566.

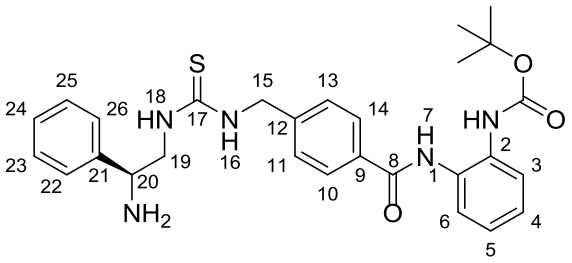
(*S*)-*N*-(2-aminophenyl)-4-(((4-phenyl-4,5-dihydro-1*H*-imidazol-2-yl)thio)methyl)benzamide (88)



To a solution of thione **87** (80 mg, 0.449 mmol) and benzyl chloride **54** (117 mg, 0.449 mmol) in acetone (5 mL) was added potassium carbonate (93 mg, 0.673 mmol) and then the mixture was stirred at reflux for 17 h. Cooled to rt and the solvent was evaporated then the residue was partitioned between water (15 mL) and ethyl acetate (30 mL), the layers were separated and the aq. layer was extracted with ethyl acetate (30 mL) then the combined organic layers were washed with brine (15 mL) dried Na_2SO_4,

and evaporated to give a beige solid (200 mg) which was purified by column chromatography on silica gel (5% methanol/ethyl acetate) to give a white solid (120 mg, 0.296 mmol, 66%). A purer sample was obtained by recrystallization from chloroform giving **88** (6 mg, 0.015 mmol, 3%) as a white solid, mp 150-151 °C; $[\alpha]_D^{25}$ -2.1 (*c* 1.0, methanol); ν_{max} (cm^{-1}) 3239 (NH), 1688 (C=N), 1642 (C=O); ^{1}H NMR (600 MHz, chloroform-*d*) δ ppm 3.58 (1H, br s, H(20)), 3.86 (2H, br s, NH_2), 4.12 (1H, br s, H(20)'), 4.39 (1H, d, *J*=13.6 Hz, H(15)), 4.44 (1H, d, *J*=13.6 Hz, H(15)'), 4.97 (1H, br s, H(19)), 6.82 - 6.89 (2H, m, ArH), 7.07 - 7.13 (1H, m, ArH), 7.22 - 7.29 (2H, m, ArH), 7.30 - 7.36 (3H, m, ArH), 7.53 (2H, d, *J*=7.9 Hz, ArH), 7.81 - 7.92 (3H, m, ArH); ^{13}C NMR (150 MHz, chloroform-*d*) δ ppm 35.2 (C(15)), 118.6 (C(3)), 120.0 (C(5)), 124.7 (C(1)), 125.2 (C(4)), 126.4 (C(11 and 13)), 127.4 (C(6)), 127.7 (C(22 and 26) and C(24)), 128.8 (C(23 and 25)), 129.6 (C(23 and 25)), 133.3 (C(9)), 140.7 (C(21)), 141.9 (C(12)), 143.4 (C(2)), 162.8 (C(17)), 165.5 (C(8)); *m/z* (ESI^+) 403 ($[M+H]^+$, 100%); HRMS $C_{23}H_{23}N_4OS^+$ requires: 403.1587, found: 403.1577.

(*S*)-*tert*-butyl (2-(4-((3-(2-amino-2-phenylethyl)thioureido)methyl)benzamido)phenyl)carbamate (89)

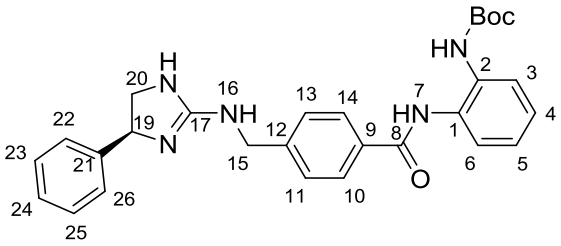


To a suspension of (*S*)-2-amino-2-phenylacetamide (1.65 g, 10.99 mmol) in anhydrous THF (20 mL) at 0 °C was added lithium aluminium hydride (2M in THF, 16.48 mL, 33.0 mmol) dropwise. The resulting solution was allowed to warm to room temperature then heated to reflux for 4 h. After cooling to 0°C, excess lithium aluminium hydride was decomposed with vigorous stirring by slow addition of potassium hydroxide (10% aq, 6 mL). The solid was removed by filtration and washed with THF (10 mL) then the filtrate was evaporated to give (*S*)-1-phenylethane-1,2-diamine as a yellow oil (2.2 g) that partially crystallized on standing overnight. The product was used without further purification despite being impure.

To a solution of impure (*S*)-1-phenylethane-1,2-diamine (213 mg, 1.565 mmol) in THF (4 mL) was added a solution of **77** (200 mg, 0.522 mmol) in THF (4 mL), the resulting solution was stirred at rt for 17.5 h, then evaporated and purified by flash column chromatography on silica gel (1-8% (2M ammonia/methanol) in dichloromethane) to give **89** (154 mg, 0.296 mmol, 57%) as a white solid, mp 105-106

°C; ν_{max} (cm^{-1}) 3276 (NH), 1689 (C=O),1660 (C=O); ^{1}H NMR (500 MHz, chloroform-*d*) δ ppm 1.47 - 1.54 (9H, m, $C(CH_3)_3$), 2.32 (4H, br s, NH_2, H(16) and H(18)), 3.44 - 3.56 (1H, m, H(19)), 3.76 (1H, br s, H(19)'), 4.12 (1H, dd, *J*=8.4, 4.3 Hz, H(20)), 4.74 (2H, br s, H(15) and H(15)'), 7.03 (1H, br s, BocNH), 7.10 - 7.20 (3H, m, ArH), 7.20 - 7.44 (7H, m, ArH), 7.59 - 7.70 (1H, m, H(6)), 7.78 (2H, d, *J*=7.7 Hz, H(10 and 14)), 9.35 (1H, br s, H(7)); ^{13}C NMR (125 MHz, chloroform-*d*) δ ppm 28.3 ($C(CH_3)_3$), 48.0 (C(15)), 51.8 (C(19)), 55.3 (C(20)), 81.5 ($OC(CH_3)_3$), 124.7 (C(3)), 125.8 (C(6) and C(5)), 126.2 (C(22 and 26)), 126.3 (C(4)), 127.7 (C(23 and 25)), 127.7 (C(10 and 14))), 127.8 (C(24)), 128.8 (C(11 and 13)), 130.4 (C(9)), 130.5 (C(2)), 132.9 (C(1)), 142.2 (C(12 and 21)), 154.7 (NCOO), 165.9 (C(8)), 183.4 (C(17)); *m/z* (ESI^+) 520 ($[M+H]^+$, 100%); HRMS $C_{28}H_{34}N_5O_3S^+$ requires: 520.2377, found: 520.2365.

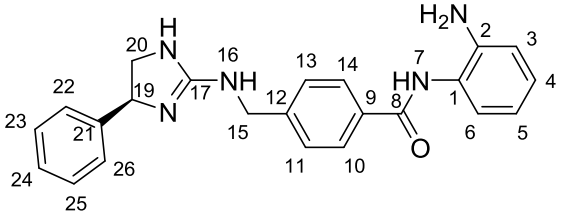
(*S*)-*tert*-butyl (2-(4-(((4-phenyl-4,5-dihydro-1*H*-imidazol-2-yl)amino)methyl)benzamido)phenyl)carbamate (90)



To a solution of thiourea **89** (154 mg, 0.296 mmol) in acetone (3 mL) was added iodomethane (0.037 mL, 0.593 mmol) and the solution stirred at rt for 18 h. Sodium bicarbonate (sat. aq., 1.5 mL) was added and the mixture was stirred for 10 min before being diluted with sodium bicarbonate (sat. aq., 20 mL) and water (5 mL), then extracted with ethyl acetate (2 × 20 mL). The combined organic layers were dried Na_2SO_4 and evaporated to give **90** (143 mg, 0.294 mmol, 99%) as a cream solid, mp 130-132 °C; ν_{max} (cm^{-1}) 3171 (NH), 1667 (C=O); ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 1.42 (9H, s, $C(CH_3)_3$), 3.35 (1H, t, *J*=7.0 Hz, H(20)), 3.84 (1H, t, *J*=8.1 Hz, H(20)'), 4.53 (2H, br s, H(15) and H(15)'), 4.86 (1H, br s, H(19)), 7.04 (1H, t, *J*=7.3 Hz, H(4)), 7.10 - 7.22 (3H, m, H(11 and 13) and H(5)), 7.22 - 7.37 (6H, m, H(23 and 25), H(24), H(22 and 26) and BocNH), 7.46 (1H, d, *J*=7.8 Hz, H(3)), 7.50 (1H, d, *J*=7.6 Hz, H(6)), 7.69 (1H, br s, C(20)NH), 7.86 (2H, d, *J*=6.6 Hz, H(10 and 14)), 8.77 (1H, br s, H(16)), 9.83 (1H, br s, H(7)); ^{13}C NMR (126 MHz, chloroform-*d*) δ ppm 28.3 ($C(CH_3)_3$), 46.3 (C(15)), 51.2 (C(20)), 58.7 (C(19)), 81.1 ($OC(CH_3)_3$), 124.8 (C(3)), 125.1 (C(6)), 126.1 (C(11 and 13)), 126.1 (C(4)), 126.4 (C(5)), 127.4 (C(10 and 14)), 128.3 (C(22 and 26)), 128.7 (C(24)), 129.1 (C(23 and 25)), 129.8 (C(9)), 131.5 (C(2)),

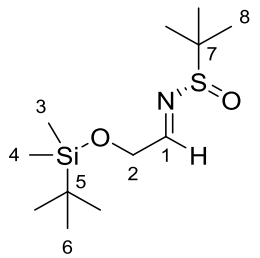
133.2 (C(1)), 139.0 (C(21)), 140.0 (C(12)), 154.5 (NCOO), 159.3 (C(17)), 166.2 (C(8)); *m/z* (ESI^+) 486 ($[M+H]^+$, 100%); HRMS $C_{28}H_{32}N_5O_3^+$ requires: 486.2500, found: 486.2496. (~90% purity by LCMS and NMR).

(*S*)-*N*-(2-aminophenyl)-4-(((4-phenyl-4,5-dihydro-1*H*-imidazol-2-yl)amino)methyl)benzamide (91)



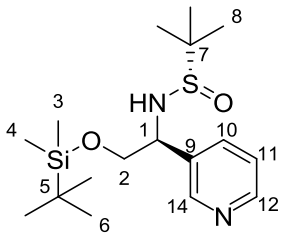
To a solution of **90** (140 mg, 0.288 mmol) in dichloromethane (3 mL) was added trifluoroacetic acid (0.444 mL, 5.77 mmol) dropwise. The resulting solution was stirred at rt for 2 h, then evaporated and the resulting white solid purified by MDAP to give **91** (52 mg, 0.135 mmol, 47%) as a white solid, mp 130-133 °C; $[\alpha]_D^{25}$ +11.1 (*c* 1.0, methanol); ν_{max} (cm^{-1}) 3217 (NH), 1672 (C=N), 1613 (C=O); ^1H NMR (400 MHz, methanol-d_4) δ ppm 3.41 (1H, dd, *J*=9.9, 7.2 Hz, H(20)), 4.02 (1H, t, *J*=9.8 Hz, H(20)'), 4.47 - 4.59 (2H, m, H(15) and H(15)'), 5.01 (1H, dd, *J*=9.5, 7.3 Hz, H(19)), 6.79 (1H, t, *J*=7.3 Hz, H(5)), 6.93 (1H, dd, *J*=8.1, 0.7 Hz, H(3)), 7.10 (1H, td, *J*=7.6, 1.2 Hz, H(4)), 7.21 (1H, d, *J*=7.6 Hz, H(6)), 7.26 - 7.41 (5H, m, PhH), 7.53 (2H, d, *J*=8.2 Hz, H(11 and 13)), 8.01 (2H, d, *J*=8.2 Hz, H(10 and 14)); ^{13}C NMR (100 MHz, methanol-d_4) δ ppm 45.7 (C(15)), 54.0 (C(20)), 61.3 (C(19)), 117.4 (C(3)), 118.3 (C(5)), 123.9 (C(1)), 125.9 (C(22 and 26)), 126.3 (C(6)), 127.0 (C(10 and 14)), 127.2 (C(4)), 127.5 (C(24)), 127.8 (C(11 and 13)), 128.4 (C(23 and 25)), 133.2 (C(9)), 142.4 (C(21) and C(2)), 142.6 (C(12)), 161.3 (C(17)), 167.2 (C(8)); *m/z* (ESI^+) 386 ($[M+H]^+$, 100%); HRMS $C_{23}H_{24}N_5O^+$ requires: 386.1975, found: 386.1974.

(*S*,*E*)-*N*-(2-((*tert*-butyldimethylsilyl)oxy)ethylidene)-2-methylpropane-2-sulfinamide (92)



A solution of 2-((*tert*-butyldimethylsilyl)oxy)acetaldehyde (2.5 g, 14.34 mmol) in dichloromethane (25 ml) and (*S*)-2-methylpropane-2-sulfinamide (1.721 g, 14.20 mmol) was stirred with copper(II) sulfate (5.15 g, 32.3 mmol) at rt for 90 h. The mixture was filtered through Celite and washed with dichloromethane, then the filtrate was concentrated and purified by flash column chromatography on silica gel (10-30% ethyl acetate/cyclohexane) to give **92** (1.665 g, 6.00 mmol, 42%) as a colourless oil; ν_{max} (cm^{-1}) 1629 (C=N); ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 0.11 (6H, s, C(3)H_3 and C(4)H_3), 0.93 (9H, s, 9 × H(6)), 1.22 (9H, s, 9 × H(8)), 4.56 (2H, d, *J*=2.9 Hz, 2 × H(2)), 8.08 (1H, s, H(1)); ^{13}C NMR (100 MHz, chloroform-*d*) δ ppm -5.3 (C(3)), -5.4 (C(4)), 18.3 (C(5)), 22.4 (C(6)), 25.8 (C(8)), 56.8 (C(7)), 65.6 (C(2)), 168.7 (C(1)); *m/z* (ESI^+) 278 ($[M+H]^+$, 100).

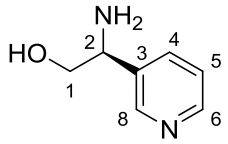
(*S*)-*N*-((*S*)-2-((*tert*-butyldimethylsilyl)oxy)-1-(pyridin-3-yl)ethyl)-2-methylpropane-2-sulfinamide (93)



To a dried 3-neck flask containing a thermometer and a stirrer bar was added anhydrous toluene (30 mL). The flask was cooled to -78 °C, BuLi (2.5 M in hexanes) (5.36 mL, 8.58 mmol) was added and the reaction mixture was stirred until the temperature returned to -78 °C. A solution of 3-bromopyridine (0.649 mL, 6.74 mmol) in anhydrous toluene (3 mL) was added to the reaction mixture a rate such that the temperature did not exceed -70 °C, and then the reaction was stirred at -78 °C for 1 hour. A solution of **92** (1.7 g, 6.13 mmol) in anhydrous toluene (2 mL) was added to the reaction mixture at a rate such that the temperature did not exceed -70 °C, and then the reaction was stirred at -78 °C for 5 hours. Methanol (1 mL) was added dropwise to quench the BuLi, then the mixture was allowed to warm to 20 ºC. Water (40 mL) was added and the mixture extracted with ethyl acetate (3 × 75 mL), the combined organics were washed with brine (50 mL), dried Na_2SO_4 and evaporated to give an orange oil (2.2 g) which was purified by flash column chromatography on silica gel (15-80% ethyl acetate/ dichloromethane) to give **93** (0.820 g, 2.300 mmol, 38%) as a colourless oil.; ν_{max} (cm^{-1}) 2926 (CH); ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 0.05 (3H, s, H(3)), 0.08 (3H, s, H(4)), 0.91 (9H, s, H(6)), 1.24 (9H, s, H(8)), 3.68 (1H, dd, *J*=10.0, 8.6 Hz,

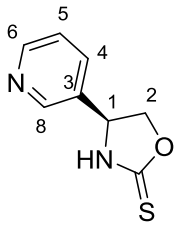
H(2)), 3.84 (1H, dd, *J*=10.0, 4.2 Hz, H(2')), 4.30 (1H, d, *J*=1.7 Hz, NH), 4.52 - 4.61 (1H, m, H(1)), 7.24 - 7.31 (1H, m, H(11)), 7.62 - 7.69 (1H, m, H(12)), 8.55 (1H, dd, *J*=4.8, 1.6 Hz, H(10)), 8.61 (1H, d, *J*=2.0 Hz, H(14)); ^{13}C NMR (100 MHz, chloroform-*d*) δ ppm -5.5 (C(3)), -5.4 (C(4)), 18.2 (C(5)), 22.6 (C(6)), 25.8 (C(8)), 55.7 (C(1)), 57.5 (C(7)), 67.3 (C(2)), 123.7 (C(11)), 135.3 (C(9)), 136.7 (C(10)), 148.2 (C(12)), 148.5 (C(14)); *m/z* (ESI$^+$) 357 ([M+H]$^+$, 100%); HRMS $C_{17}H_{33}N_2O_2SSi^+$ requires: 357.2027, found: 357.2026.

(*S*)-2-amino-2-(pyridin-3-yl)ethanol (94)



To a solution of sulfinamide **93** (0.816 g, 2.288 mmol) in methanol (10 mL) was added 4M HCl in dioxane (2.86 mL, 11.44 mmol) at 0 °C. The mixture was stirred at 25 rtC for 5 h. The mixture was evaporated and the resulting oil was dissolved in methanol and loaded onto an SCX-2 cartridge. It was washed with methanol (20 mL) and then eluted with 2M ammonia in methanol (50 mL) which was evaporated to give the free base **94** (310 mg, 2.244 mmol, 98%) as a yellow oil; ν_{max} (cm^{-1}) 3270 (NH/OH); ^{1}H NMR (400 MHz, methanol-*d*$_4$) δ ppm 3.68 (1H, dd, *J*=11.0, 7.0 Hz, H(1)), 3.75 (1H, dd, *J*=11.0, 5.1 Hz, H(1')), 4.09 (1H, dd, *J*=7.0, 5.1 Hz, H(2)), 7.45 (1H, dd, *J*=7.8, 4.9 Hz, H(5)), 7.91 (1H, d, *J*=8.1 Hz, H(4)), 8.47 (1H, dd, *J*=4.9, 1.5 Hz, H(6)), 8.59 (1H, d, *J*=2.2 Hz, H(8)); ^{13}C NMR (100 MHz, methanol-*d*$_4$) δ ppm 54.9 (C(1)), 66.2 (C(2)), 123.8 (C(5)), 135.5 (C(4)), 138.0 (C(3)), 147.7 (C(6)), 147.9 (C(8)); *m/z* (EI$^+$) 139 ([M+H]$^+$, 100%); HRMS $C_7H_{11}N_2O^+$ requires: 139.0866, found: 139.0862.

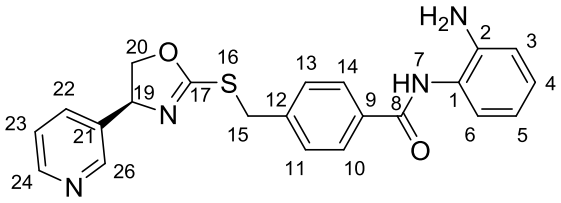
(*S*)-4-(pyridin-3-yl)oxazolidine-2-thione (95)



To a stirring mixture of **94** (160 mg, 1.158 mmol), potassium carbonate (80 mg, 0.579 mmol) and carbon disulfide (0.140 mL, 2.316 mmol) in ethanol (1 mL) at 50 °C was added hydrogen peroxide (0.152 mL, 1.737 mmol) dropwise [Caution: Exotherm! - use a blast shield]. After completion of addition the mixture was cooled to rt and

filtered. The filtrate was diluted with ethyl acetate (30 ml) washed with water (2 × 15 ml), sodium sulfite (10%, 15 mL) and brine, then dried Na_2SO_4 and evaporated to give a yellow solid (57 mg). Purification by flash column chromatography on silica gel (0-5% (2M ammonia/methanol) in dichloromethane) gave **95** (29 mg, 0.161 mmol, 14%) as a white solid, mp 157-159 °C; ν_{max} (cm^{-1}) 3083 (NH); ^{1}H NMR (400 MHz, methanol-d_4) δ ppm 4.52 (1H, dd, *J*=9.2, 6.2 Hz, H(2)), 5.04 (1H, t, *J*=9.2 Hz, H(2)'), 5.28 (1H, dd, *J*=9.2, 6.2 Hz, H(1)), 7.53 (1H, dd, *J*=7.8, 4.9 Hz, H(5)), 7.87 (1H, d, *J*=7.8 Hz, H(4)), 8.49 - 8.63 (2H, m, H(6) and H(8)); ^{13}C NMR (100 MHz, methanol-d_4) δ ppm 57.6 (C(1)), 76.8 (C(2)), 124.4 (C(4)), 134.8 (C(4)), 136.0 (C(3)), 147.2 (C(6)), 149.1 (C(8)), 190.2 (C=S); *m/z* (ESI^+) 181 ($[M+H]^+$, 100%); HRMS $C_8H_9N_2S^+$ requires:181.0436, found: 181.0430.

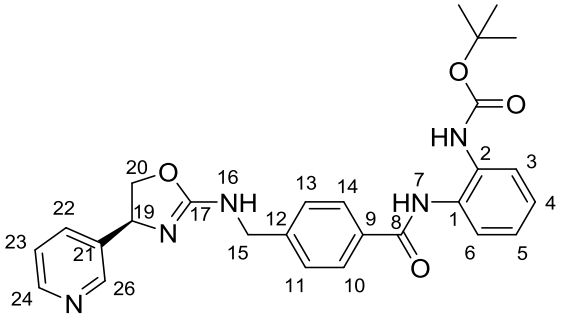
(*S*)-*N*-(2-aminophenyl)-4-(((4-(pyridin-3-yl)-4,5-dihydrooxazol-2-yl)thio)methyl)benzamide (96)



To a solution of **95** (25 mg, 0.139 mmol) and benzyl chloride **54** (36.2 mg, 0.139 mmol) in acetone (2 mL) was added potassium carbonate (28.8 mg, 0.208 mmol) and then the mixture was stirred at reflux for 17 h. The reaction had dried out overnight so acetone (3 mL) was added and then stirred at reflux for 6 h. Cooled to rt and partitioned between water (15 ml) and ethyl acetate (20 ml), the aq. layer was extracted with ethyl acetate (15 ml) then the combined organics were dried Na_2SO_4, and evaporated to give a yellow solid (57 mg) which was purified by MDAP to give **96** (25 mg, 0.062 mmol, 45%) as a white solid, mp 107-110 °C; $[\alpha]_D^{25}$ +22.0 (*c* 0.25, methanol); ν_{max} (cm^{-1}) 3212 (NH), 1645 (C=O); ^{1}H NMR (500 MHz, methanol-d_4) δ ppm 4.23 (1H, t, *J*=8.0 Hz, H(20)), 4.38 (1H, d, *J*=13.7 Hz, H(15)), 4.44 (1H, d, *J*=13.7 Hz, H(15)'), 4.83 (1H, t, *J*=9.3 Hz, H(20)'), 5.35 (1H, dd, *J*=9.3, 7.7 Hz, H(19)), 6.80 (1H, t, *J*=7.4 Hz, H(5)), 6.93 (1H, d, *J*=8.0 Hz, H(3)), 7.11 (1H, t, *J*=7.4 Hz, H(4)), 7.22 (1H, d, *J*=7.7 Hz, H(6)), 7.43 (1H, dd, *J*=7.7, 4.9 Hz, H(23)), 7.60 (3H, d, *J*=8.0 Hz, H(11 and 13) and H(22), 7.96 (2H, d, *J*=8.0 Hz, H(10 and 14)), 8.40 (1H, s, H(26)), 8.49 (1H, d, *J*=4.7 Hz, H(24); ^{13}C NMR (125 MHz, methanol-d_4) δ ppm 34.8 (C(15)), 67.1 (C(19)), 75.9 (C(20)), 117.3 (C(3)), 118.3 (C(5)), 123.9 (C(23)), 124.1 (C(1)), 126.3 (C(6)), 127.2 (C(4)), 127.7 (C(10 and 14)), 128.9 (C(11 and 13)), 133.4 (C(9)), 135.1 (C(22)), 138.3

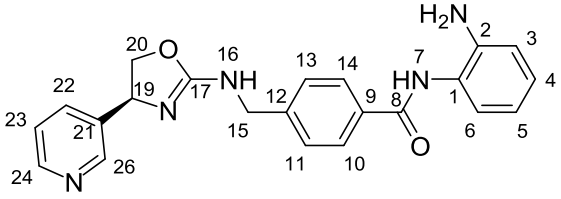
(C(21)), 141.5 (C(2)), 142.4 (C(12)), 147.2 (C(26)), 148.0 (C(24)), 167.1 (C(17)), 167.7 (C(8)); *m/z* (ESI^+) 405 ($[M+H]^+$, 100%); HRMS $C_{22}H_{21}N_4O_2S^+$ requires: 405.1380, found: 405.1384; 93% purity by LCMS.

(*S*)-*tert*-butyl (2-(4-(((4-(pyridin-3-yl)-4,5-dihydrooxazol-2-yl)amino)methyl)benzamido)phenyl)carbamate (97)



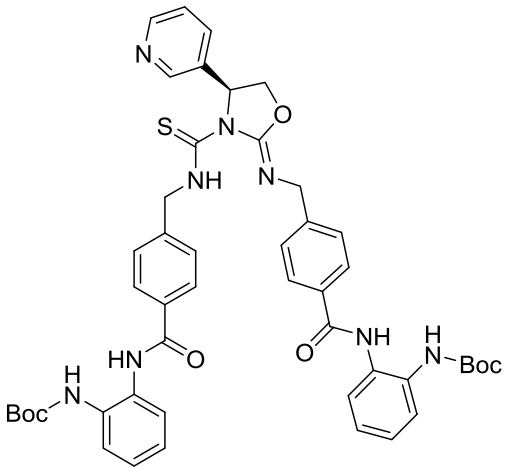
To a solution of **94** (155 mg, 1.119 mmol) in THF (8 mL) and methanol (3 mL) was added isothiocyanate **77** (330 mg, 0.861 mmol), the solution was stirred at rt for 17 h, and then solvents were evaporated. The residue was dissolved in acetone (4 mL), then iodomethane (0.108 mL, 1.721 mmol) was added and the solution stirred at rt for 5 h. Sodium bicarbonate (sat. aq., 2 mL) was added, stirred for 10 min then diluted with sodium bicarbonate (sat. aq., 20 mL), and extracted with ethyl acetate (3 × 30 mL). The combined organic layers were washed with brine (20 mL), dried Na_2SO_4 and evaporated to give a cream solid (370 mg). Purification by flash column chromatography on silica gel (2-10% (2M ammonia/methanol) in dichloromethane) gave **97** (86 mg, 0.176 mmol, 21%) as a white solid, mp 107-108 °C; ν_{max} (cm^{-1}) 3265 (NH), 1651 (C=O); ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 1.50 (9H, s, $C(CH_3)_3$), 4.06 (1H, dd, *J*=7.8, 7.2 Hz, H(19)), 4.48 (2H, s, H(15) and H(15)'), 4.66 (1H, dd, *J*=7.8, 7.0 Hz, H(20)), 5.12 (1H, dd, *J*=8.7, 7.2 Hz, H(20)'), 5.43 (1H, br s, H(16)), 7.09 - 7.22 (2H, m, ArH and NHBoc), 7.22 - 7.30 (1H, m, ArH), 7.30 - 7.42 (4H, m, ArH), 7.56 (1H, d, *J*=7.8 Hz, ArH), 7.73 (1H, d, *J*=6.8 Hz, ArH), 7.88 (2H, d, *J*=7.8 Hz, H(10 and 14)), 8.42 (1H, s, H(26)), 8.49 (1H, d, *J*=3.7 Hz, H(24)), 9.39 (1H, br s, H(7)); ^{13}C NMR (100 MHz, chloroform-*d*) δ ppm 28.3 ($C(CH_3)_3$), 46.6 (C(15)), 65.5 (C(19)), 74.9 (C(20)), 81.1 ($OC(CH_3)_3$), 123.6 (C(23)), 124.5 (C(3)), 125.6 (C(6)), 125.7 (C(4)), 126.0 (C(5)), 127.4 (C(11 and 13)), 127.8 (C(10 and 14)), 130.3 (C(9)), 130.7 (C(2)), 133.5 (C(1)), 134.2 (C(22)), 139.2 (C(21)), 142.6 (C(12)), 148.2 (C(24)), 148.7 (C(26)), 154.6 (NCOO), 162.0 (C(17)), 165.7 (C(8)); *m/z* (ESI^+) 488 ($[M+H]^+$, 100%); HRMS $C_{27}H_{30}N_5O_4^+$ requires: 488.2292, found: 488.2285.

(*S*)-*N*-(2-aminophenyl)-4-(((4-(pyridin-3-yl)-4,5-dihydrooxazol-2-yl)/amino)methyl)benzamide (98)



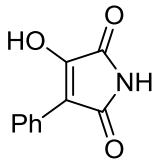
To a solution of **97** (80 mg, 0.164 mmol) in dichloromethane (1 mL) was added trifluoroacetic acid (0.253 mL, 3.28 mmol). The resulting solution was stirred at rt for 1.5 h, then quenched by pouring onto sodium bicarbonate (sat. aq., 10 mL) and extracted with ethyl acetate (3 × 10 mL). The combined organics were washed with brine, dried Na_2SO_4, and evaporated to give a yellow solid (60 mg) which was purified by flash column chromatography on silica gel (2-10% (2M ammonia/methanol) in dichloromethane) to give to give **98** (38 mg, 0.098 mmol, 60%) as a white solid, mp 89-90 °C (dec.); $[\alpha]_D^{25}$ -20.0 (*c* 1.0, methanol); ν_{max} (cm^{-1}) 3214 (NH), 1652 (C=O); ^{1}H NMR (400 MHz, methanol-d_4) δ ppm 4.12 (1H, dd, *J*=8.3, 6.6 Hz, H(20)), 4.42 - 4.58 (2H, m, H(15) and H(15)'), 4.73 (1H, dd, *J*=9.1, 8.3 Hz, H(20)'), 5.16 (1H, dd, *J*=9.1, 6.6 Hz, H(19)), 6.79 (1H, td, *J*=7.6, 1.0 Hz, H(5)), 6.93 (1H, dd, *J*=8.1, 1.0 Hz, H(3)), 7.10 (1H, td, *J*=7.6, 1.3 Hz, H(4)), 7.22 (1H, d, *J*=7.6 Hz, H(6)), 7.41 (1H, dd, *J*=7.8, 5.1 Hz, H(23), 7.53 (2H, d, *J*=8.1 Hz, H(11 and 13)), 7.70 (1H, dt, *J*=7.8, 1.8 Hz, H(22)), 8.00 (2H, d, *J*=8.1 Hz, H(10 and 14)), 8.41 - 8.49 (2H, m, H(24) and H(26); ^{13}C NMR (100 MHz, methanol-d_4) δ ppm 45.5 (C(15)), 64.5 (C(19)), 74.6 (C(20)), 117.3 (C(3)), 118.3 (C(5)), 123.9 (C(23)), 126.3 (C(6)), 126.9 (C(10 and 14)), 127.1 (C(4)), 127.7 (C(11 and 13)), 127.7 (C(1)), 133.1 (C(9)), 135.0 (C(22)), 140.1 (C(21)), 142.4 (C(2)), 143.3 (C(12)), 147.2 (C(26)), 147.7 (C(24)), 163.3 (C(17)), 167.3 (C(8)); *m/z* (ESI^+) 388 ($[M+H]^+$, 100%); HRMS $C_{22}H_{22}N_5O_2^+$ requires: 388.1768, found: 388.1774.

(*S*,*Z*)-*tert*-butyl (2-(4-((2-((*tert*-butoxycarbonyl)amino)phenyl)carbamoyl)benzyl)imino)-4-(pyridin-3-yl)oxazolidine-3-carbothioamido)methyl)benzamido)phenyl)carbamate (99)



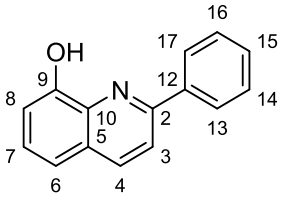
To a solution of **94** (79 mg, 0.572 mmol) in THF (3 mL) was added a solution of **77** (230 mg, 0.600 mmol) in THF (3 mL), the solution was then stirred at rt for 20 h, solvents were evaporated and the residue dissolved in Acetone (3 mL), then iodomethane (0.072 mL, 1.144 mmol) was added and the solution stirred at rt for 21 h. Sodium bicarbonate (sat. aq., 2 mL) was added, stirred for 10 min then diluted with sodium bicarbonate (sat. aq., 20 mL), and extracted with ethyl acetate (2 × 20 mL). Combined organics were washed with brine (20 mL), dried Na_2SO_4 and evaporated to give 240 mg of a yellow solid, which was purified by flash column chromatography on silica gel (0.2 to 3% (2M ammonia/methanol) in dichloromethane) to give **99** (47 mg, 0.054 mmol, 9%) as a white solid, 87-90 °C; ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 1.47 (9H, s), 1.49 (9H, s), 1.93 (1H, br s), 4.35 (1H, dd, *J*=8.3, 1.8 Hz), 4.53 (1H, d, *J*=17.0 Hz), 4.62 (1H, d, *J*=17.0 Hz), 4.65 (1H, dd, *J*=14.8, 3.8 Hz), 4.75 (1H, t, *J*=8.3 Hz), 4.86 (1H, dd, *J*=14.8, 4.5 Hz), 6.26 (1H, dd, *J*=8.3, 1.8 Hz), 6.95 - 7.08 (5H, m), 7.09 - 7.19 (3H, m), 7.35 (1H, dd, *J*=8.1, 4.9 Hz), 7.37 - 7.48 (4H, m), 7.51 (1H, d, *J*=7.8 Hz), 7.60 (1H, d, *J*=7.6 Hz), 7.67 (1H, dt, *J*=7.9, 1.9 Hz), 7.80 (2H, d, *J*=8.1 Hz), 7.91 (2H, d, *J*=8.1 Hz), 8.60 (1H, dd, *J*=4.8, 1.6 Hz), 8.65 (1H, d, *J*=2.2 Hz), 9.30 (1H, br s), 9.45 (1H, br s), 12.14 (1H, t, *J*=3.8 Hz); ^{13}C NMR (100 MHz, chloroform-*d*) δ ppm 28.3, 28.4, 50.1, 50.1, 60.0, 71.5, 80.5, 81.3, 123.8, 124.5, 125.1, 125.3, 125.8, 125.8, 126.0, 126.2, 127.0, 127.8, 128.2, 128.9, 129.9, 130.1, 130.4, 131.0, 132.5, 134.1, 134.2, 135.6, 140.3, 143.6, 148.2, 149.6, 150.2, 154.3, 154.7, 165.8, 166.0, 177.5; *m/z* (ESI$^+$) 871 ([M+H]$^+$, 100%); HRMS $C_{47}H_{51}N_8O_7^+$ requires: 871.3596, found: 871.3587.

3-hydroxy-4-phenyl-1*H*-pyrrole-2,5-dione (100)[127]



To a solution of diethyl oxylate (1 mL, 7.40 mmol) and 2-phenylacetamide (0.5 g, 3.70 mmol) in anh. THF (11 mL) at 0 °C was added a solution of potassium *tert*-butoxide (1.04 g, 9.25 mmol) in anh. THF (9 mL). The mixture was stirred at 0 °C for 1.5 h then quenched with hydrochloric acid (0.5 M, aq, 20 mL) and extracted with ethyl acetate (2 × 20 mL). The combined organic layers were washed with brine (20 mL), dried Na_2SO_4 and evaporated to give a yellow oil that was triturated with diethyl ether to give a white solid which was unreacted 2-phenylacetamide. The combined filtrates from trituration were evaporated and the residue purified by column chromatography on silica gel (ethyl acetate:hexane, 4:1) to give **100** as a yellow solid (26 mg, 0.137 mmol, 4%), mp 152-158 °C (lit.[127] 214-216 °C); ν_{max} (cm^{-1}) 3348 (OH), 3159 (NH), 1632 (C=O); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 6.93 (1H, t, *J*=6.9 Hz, *p*-Ph), 7.19 (2H, t, *J*=7.6 Hz, *m*-Ph), 8.16 (2H, d, *J*=7.6 Hz, *o*-Ph), 9.49 (1H, br s, NH). [15% impurity by ^{1}H NMR].

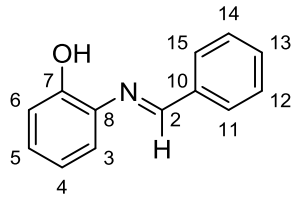
2-phenylquinolin-8-ol (101)[128]



In an oven-dried flask was added lithium (83 mg, 12 mmol) and anh. diethyl ether (5 mL) then bromobenzene (0.6 mL, 5.7 mmol) dropwise under argon. An exotherm was observed and the solvent began to reflux, the mixture was stirred without heating for 1 h, then a solution of 8-hydroxyquinoline (0.4 g, 2.75 mmol) in anh. diethyl ether (8 mL) was added slowly and the mixture heated to reflux for 1 h. The reaction was cooled to rt and air was bubbled through the mixture for 2 h, then water (4 mL) and diethyl ether (8 mL) were added and the mixture was neutralised with hydrochloric acid (1 M, aq.). The layers were separated and the aqueous layer was extracted with diethyl ether (2 × 25 mL). The combined organic layers were dried Na_2SO_4, and evaporated to give a brown oil. Purification by column chromatography on silica gel (ethyl

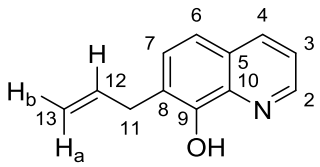
acetate:hexane, 1:19) gave **101** as a white solid (0.424 g, 1.92 mmol, 70 %), mp 55-57 °C (lit.[163] 55 °C); ν_{max} (cm^{-1}) 3385 (OH); ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 7.23 (1H, d, *J*=7.3 Hz, H(8)), 7.36 (1H, d, *J*=8.0 Hz, H(3)), 7.42 - 7.60 (4H, m, H(7), H(15) and H(13 and 17)), 7.92 (1H, d, *J*=8.8 Hz, H(6)), 8.18 (2H, d, *J*=7.0 Hz, H(14 and 16)), 8.23 (1H, d, *J*=8.5 Hz, H(4)), 8.42 (1H, br s, OH); ^{13}C NMR (125 MHz, chloroform-*d*) δ ppm 110.2 (C(8)), 117.6 (C(6)), 119.5 (C(3)), 127.3 (C(5)), 127.4 (C(13 and 17)), 127.5 (C(15)), 128.8 (C(14 and 16)), 129.6 (C(7)), 137.0 (C(4)), 137.9 (C(10)), 138.7 (C(12)), 152.3 (C(9)), 154.9 (C(2)).

(*E*)-2-(benzylideneamino)phenol (102)[129]



To a solution of 2-hydroxyaniline (0.537 g, 4.92 mmol) in methanol (3 mL) was added slowly a solution of benzaldehyde (0.5 mL, 4.92 mmol) in methanol (1.5 mL). The solution was stirred at reflux for 3 h, then cooled and placed in the refrigerator overnight. A brown solid was filtered and then recrystallized from ethanol to give **102** as a grey solid (0.360 g, 1.82 mmol, 37%), mp 90-91 °C (lit.[129] 87 °C); ν_{max} (cm^{-1}) 3323 (OH), 1624 (C=N); ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 6.95 (1H, td, *J*=7.7, 1.3 Hz, H(3)), 7.06 (1H, dd, *J*=8.2, 1.4 Hz, H(6)), 7.24 (1H, td, *J*=7.8, 1.5 Hz, H(4)), 7.31 (1H, br s, OH), 7.34 (1H, dd, *J*=8.0, 1.3 Hz, H(5)), 7.48 - 7.57 (3H, m, H(12 and 14) and H(13)), 7.91 - 8.00 (2H, m, H(11 and 15)), 8.73 (1H, s, H(2)); ^{13}C NMR (125 MHz, chloroform-*d*) δ ppm 115.0 (C(3)), 115.8 (C(6)), 120.1 (C(4)), 128.8 (C(12 and 14)), 128.8 (C(11 and 15)), 128.9 (C(5)), 131.6 (C(13)), 135.5 (C(10)), 135.8 (C(8)), 152.3 (C(7)), 157.1 (C(2)).

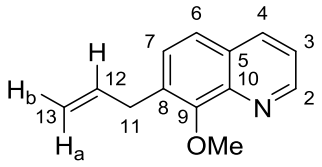
7-allyl-8-hydroxyquinoline (103)[164]



Acetone (100 mL) was stirred over $MgSO_4$ (2 g) for 10 min then filtered into an oven-dried flask. To this flask was added 8-hydroxyquinoline (10 g, 68.9 mmol) and potassium carbonate (14.28 g, 103.4 mmol), the mixture was stirred at reflux under a

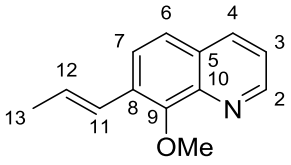
nitrogen atmosphere for 15 min then allyl bromide (6.2 mL, 71.7 mmol) was added and the mixture stirred at reflux for 24 h. After cooling the reaction was filtered, the solids were washed with acetone (3 × 30 mL) and the filtrate was evaporated. The residue was dissolved in diethyl ether (80 mL), washed with potassium hydroxide (5% aq., 80 mL) and water (2 × 80 mL), then dried Na_2SO_4 and evaporated to give a dark brown oil. The oil was stirred and heated at 200 °C for 30 min then cooled to rt and purified by column chromatography on silica gel (hexane:ethyl acetate, 3:1) to give a dark brown solid (11.71 g, 63.2 mmol, 92%), mp 40 – 44 °C (lit.[164] 42 °C (n-heptane)); ν_{max} (cm^{-1}) 3372 (OH); ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 3.68 (2H, dt, *J*=6.5, 1.4 Hz, H(11)), 5.16 (1H, dq, *J*=27.9, 1.5 Hz, H_a(13)), 5.14 - 5.16 (1H, m, H, H_b(13)), 6.12 (1H, ddt, *J*=16.9, 10.2, 6.5, 6.5 Hz, H(12)), 7.32 (1H, d, *J*=8.8 Hz, H(7)), 7.37 - 7.43 (2H, m, H(8) and H(3)), 8.14 (1H, dd, *J*=8.3, 1.5 Hz, H(4)), 8.47 (1H, br s, OH), 8.79 (1H, dd, *J*=4.3, 1.5 Hz, H(2)).

7-allyl-8-methoxyquinoline (104)



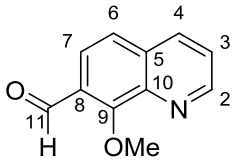
To a solution of the hydroxyl compound **103** (3 g, 16.2 mmol) in THF (100 mL) was added powdered potassium hydroxide (3.91 g, 69.7 mmol) followed by iodomethane (1.51 mL, 24.3 mmol). The mixture was stirred at rt for 18 h then inorganics were filtered and the filtrate evaporated to give **104** as a brown oil (3.23 g, 16.2 mmol, 100%); ν_{max} (cm^{-1}) 1638 (C=C); *m/z* (ESI$^+$) 200 ($[M+H]^+$, 100%); ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 3.66 (2H, d, *J*=6.5 Hz, H(11)), 4.13 (3H, s, OCH_3), 5.04 - 5.18 (2H, m, H_b(13) and H(12)), 6.05 (1H, ddt, *J*=16.8, 10.3, 6.5, H_a(13)), 7.31 - 7.45 (2H, m, H(7)) and H(3), 7.53 (1H, d, *J*=8.5 Hz, H(8)), 8.12 (1H, dd, *J*=8.2, 1.6 Hz, H(4)), 8.93 (1H, dd, *J*=4.1, 1.6 Hz, H(2)); ^{13}C NMR (125 MHz, chloroform-*d*) δ ppm 34.2 (C(11)), 62.4 (OCH_3), 116.0 (C(13)), 120.6 (C(6)), 123.0 (C(3)), 128.6 (C(8)), 128.8 (C(7)), 132.3 (C(5)), 136.0 (C(4)), 136.9 (C(12)), 142.9 (C(10)), 149.5 (C(2)), 153.4 (C(9)); HRMS $C_{13}H_{14}NO^+$ requires: 200.1070, found: 200.1074.

(*E*)-8-methoxy-7-(prop-1-en-1-yl)quinoline (106)



To a solution of the allyl compound **104** (3.0 g, 15.05 mmol) in anhydrous THF (130 mL) was added potassium *tert*-butoxide (6.75 g, 60.2 mmol) under a nitrogen atmosphere. The suspension was stirred at rt for 2 h then poured onto ammonium chloride (sat. aq., 50 mL) and extracted with diethyl ether (2 × 150 mL). The combined organic layers were washed with brine (100 mL), dried Na_2SO_4 and evaporated to give **106** as a brown oil (2.82 g, 14.1 mmol, 94%); ν_{max} (cm^{-1}) 1609 (C=C); ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 2.01 (3H, dd, *J*=6.7, 1.6 Hz, H(13)), 4.11 (3H, s, OCH_3), 6.44 (1H, dq, *J*=15.9, 6.7 Hz, H(12)), 7.02 (1H, dq, *J*=15.9, 1.6 Hz, H(11)), 7.35 (1H, dd, *J*=8.2, 4.3 Hz, H(3)), 7.51 (1H, d, *J*=8.8 Hz, H(7)), 7.69 (1H, d, *J*=8.8 Hz, H(6)), 8.10 (1H, dd, *J*=8.2, 1.6 Hz, H(4)), 8.93 (1H, dd, *J*=4.3, 1.6 Hz, H(2)); ^{13}C NMR (125 MHz, chloroform-*d*) δ ppm 19.1 (C(13)), 62.5 (OCH_3), 120.7 (C(6)), 123.3 (C(3)), 124.4 (C(7)), 125.3 (C(11)), 128.3 (C(12)), 128.8 (C(5)), 130.2 (C(8)), 136.0 (C(4)), 143.4 (C(10)), 149.8 (C(2)), 152.1 (C(9)); *m/z* (ESI^+) 200 ($[M+H]^+$, 100%); HRMS $C_{13}H_{14}NO^+$ requires: 200.1070, found: 200.1075.

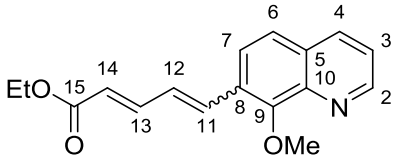
8-methoxyquinoline-7-carbaldehyde (107)



To a solution of the allyl compound **104** (3.17 g, 15.9 mmol) in dioxane (200 mL) and water (80 mL) was added osmium tetroxide (4% in water, 1.1 mL, 0.159 mmol) and then stirred at rt for 15 min. Sodium (meta)periodate (17 g, 79.5 mmol) was added portionwise over 30 min and the reaction was stirred at rt for 16 h. Sodium thiosulfate (18 g) was added and the reaction poured onto water (500 mL), stirred for 30 min then the insoluble material was filtered and the dioxane was evaporated. The remaining aqueous solution was extracted with chloroform (2 × 150 mL) and ethyl acetate (2 × 100 mL), both chlorinated and non-chlorinated organic layers were separately washed with brine (100 mL), then combined and dried Na_2SO_4 and evaporated to give **107** as a beige solid (2.59 g, 13.8 mmol, 87%), mp 86-88 °C; ν_{max}

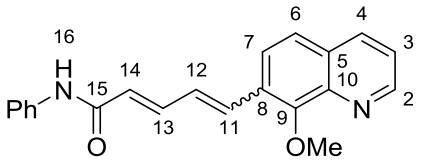
(cm^{-1}) 1671 (C=O); ^{1}H NMR (500 MHz, chloroform-*d*) δ ppm 4.39 (3H, s, OCH_3), 7.52 (1H, dd, *J*=8.3, 4.1 Hz, H(3)), 7.58 (1H, d, *J*=8.7 Hz, H(7)), 7.91 (1H, d, *J*=8.7 Hz, H(6)), 8.17 (1H, dd, *J*=8.3, 1.7 Hz, H(4)), 8.99 (1H, dd, *J*=4.1, 1.7 Hz, H(2)), 10.68 (1H, d, *J*=0.8 Hz, H(11)): ^{13}C NMR (125 MHz, chloroform-*d*) δ ppm 64.9 (OCH_3), 123.4 (C(6)), 123.5 (C(7)), 123.6 (C(3)), 127.6 (C(5)), 133.6 (C(8)), 136.5 (C(4)), 143.1 (C(10)), 150.1 (C(2)), 161.7 (C(9)), 190.2 (C(11)); *m/z* (ESI^+) 188 ($[M+H]^+$, 95%); HRMS $C_{11}H_{10}NO_2^+$ requires: 188.0706, found: 180.0705.

ethyl (2*E*)-5-(8-methoxyquinolin-7-yl)penta-2,4-dienoate (113)



To a flame dried flask was added arsonium salt **121** (515 mg, 1.03 mmol) and anhydrous THF (4 mL) under a nitrogen atmosphere. The suspension was cooled to 0 °C then *n*-butyllithium (2.3 M in hexanes, 0.45 mL, 1.03 mmol) was added dropwise and stirred for 15 min at 0 °C then 15 min at rt. The aldehyde **107** (175 mg, 0.94 mmol) was then added portion-wise over 5 min under a nitrogen atmosphere and stirring continued for 16 h at 8rt. The reaction was then concentrated to give an orange solid which was purified by column chromatography on silica gel (ethyl acetate:hexane, 1:1) to give **113** as a yellow oil that crystallised on standing [a mixture of *E* and *Z* isomers] (200 mg, 0.706 mmol, 75%). The product was not purified further before hydrogenation.

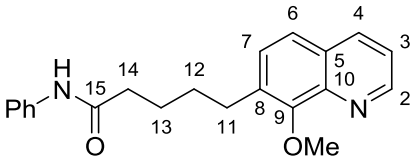
(2*E*)-5-(8-methoxyquinolin-7-yl)-*N*-phenylpenta-2,4-dienamide (114)



To a suspension of **122** (140 mg, 0.55 mmol) in dichloromethane (2 mL) was added oxalyl chloride (0.066 mL, 0.78 mmol) followed by 1 drop of DMF. Stirred at rt for 1 h, and at reflux for 1 h, then evaporated and the residue was suspended in dichloromethane (2 mL). To this suspension was added aniline (0.047 mL, 0.52 mmol) and pyridine (1 mL), the resulting solution was stirred at rt for 16 h. Quenched with sodium hydrogen carbonate (sat. aq., 2 mL) and extracted with chloroform (2 × 25 mL), the combined organic layers were washed with water (2 × 10 mL), brine (10 mL) then

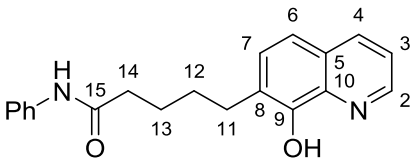
dried Na_2SO_4 and evaporated. Partial purification by column chromatography on silica gel (ethyl acetate:hexane, 2:1) gave **114** as a mixture of *E* and *Z* isomers (65 mg, 0.197 mmol, 38%) which was not purifier further before hydrogenation.

5-(8-methoxyquinolin-7-yl)-*N*-phenylpentanamide (116)



To ethyl ester **125** (35 mg, 0.122 mmol) was added a solution of aniline (12 mg, 0.122 mmol) in anhydrous toluene (1 mL) under a nitrogen atmosphere. To the resulting solution was added trimethylaluminium (1M in heptane, 0.12 mL, 0.122 mmol) and then stirred at rt for 1 h. Another portion of trimethylaluminium (1M in heptane, 0.037 mL, 0.37 mmol) was added and the reaction stirred at rt for 16 h. Quenched carefully with ammonium chloride (sat. aq., 5 mL) then diluted with ethyl acetate (25 mL) and water (20 mL). The aqueous layer was extracted with ethyl acetate (25 mL), and the combined organic layers were then washed with brine (20 mL), dried Na_2SO_4 and evaporated. Purification by column chromatography on silica gel (ethyl acetate:hexane, 3:2) gave **116** as a white solid (25 mg, 0.0748 mmol, 61%), mp 136-137 °C; ν_{max} (cm^{-1}) 3250 (NH), 1686 (C=O); ^{1}H NMR (500 MHz, chloroform-*d*) δ ppm 1.63 - 1.92 (4H, m, H(12) and H(13)), 2.35 - 2.47 (2H, m, H(11)), 2.87 - 2.99 (2H, m, H(14)), 4.14 (3H, s, OCH_3), 7.06 (1H, t, *J*=7.4 Hz, *p*-Ph), 7.29 (2H, t, *J*=7.8 Hz, *m*-Ph), 7.33 - 7.40 (2H, m, H(3) and H(7)), 7.48 - 7.62 (4H, m, *o*-Ph, H(6) and NH), 8.11 (1H, dd, *J*=8.2, 1.4 Hz, H(4)), 8.92 (1H, dd, *J*=4.0, 1.5 Hz, H(2)); ^{13}C NMR (125 MHz, chloroform-*d*) δ ppm 25.3 (C(11)), 29.4 (C(12)), 30.1 (C(13)), 37.5 (C(14)), 62.5 (OCH_3), 119.8 (*m*-Ph), 120.7 (C(6)), 123.3 (C(3)), 124.0 (C(7)), 128.6 (C(5)), 128.9 (*p*-Ph), 129.0 (*o*-Ph), 134.5 (C(8)), 136.1 (C(4)), 138.3 (*i*-Ph), 142.9 (C(10)), 149.6 (C(2)), 153.4 (C(9)), 171.5 (C(15)); *m/z* (ESI$^+$) 335 ([M+H]$^+$, 100%); HRMS $C_{21}H_{23}N_2O_2^+$ requires: 335.1754, found: 335.1759.

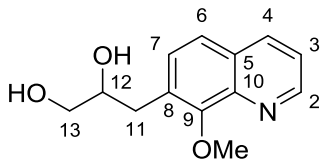
5-(8-hydroxyquinolin-7-yl)-*N*-phenylpentanamide (117)



To a solution of **116** (36 mg, 0.12 mmol) in dichloromethane (1 ml) at -10 °C

was added boron tribromide (1M in dichloromethane, 0.35 ml, 0.35 mmol) dropwise. The resulting mixture was stirred at -10 °C for 5 min then warmed to rt and stirred for 2 h. Quenched with sodium hydrogen carbonate (sat. aq., 2 ml), diluted with water (15 ml) and chloroform (25 ml). The aqueous layer was extracted with chloroform (25 mL) and the combined organic layers were washed with brine (20 mL) then dried Na_2SO_4 and evaporated to give a yellow solid (30 mg). Purification by column chromatography on silica gel (ethyl acetate:hexane, 1:1) gave **117** as a white solid (19 mg, 0.059 mmol, 49%), mp 157-159 °C; ν_{max} (cm^{-1}) 3302 (OH), 1653 (C=O); ^{1}H NMR (600 MHz, methanol-d_4) δ ppm 1.74 - 1.84 (4H, m, H(12) and H(13)), 2.42 (2H, t, *J*=6.8 Hz, H(11)), 2.90 (2H, t, *J*=7.0 Hz, H(14)), 7.06 (1H, t, *J*=7.3 Hz, *p*-Ph), 7.28 (2H, t, *J*=7.9 Hz, *m*-Ph), 7.31 (1H, d, *J*=8.5 Hz, H(7)), 7.37 (1H, d, *J*=8.5 Hz, H(6)), 7.42 (1H, dd, *J*=8.3, 4.1 Hz, H(3)), 7.52 (2H, d, *J*=8.3 Hz, *o*-Ph), 8.19 (1H, dd, *J*=8.3, 1.1 Hz, H(4)), 8.76 (1H, dd, *J*=4.1, 1.1 Hz, H(2)); ^{13}C NMR (150 MHz, methanol-d_4) δ ppm 26.8 (C(11)), 30.6 (C(12)), 30.7 (C(13)), 37.9 (C(14)), 118.4 (C(6)), 121.3 (*o*-Ph), 122.0 (C(3)), 125.1 (C(7)), 125.4 (C(5)), 128.6 (C(8)), 129.7 (*m*-Ph), 130.8 (C(*p*-Ph)), 137.1 (C(4)), 139.7 (C(*i*-Ph)), 139.9 (C(10)), 149.2 (C(2)), 150.9 (C(9)), 174.7 (C(15)); *m/z* (ESI^+) 321 ($[M+H]^+$, 100%); HRMS $C_{20}H_{21}N_2O_2^+$ requires: 321.1598, found: 321.1601.

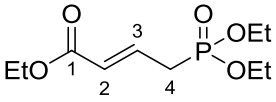
7-((2,3-dihydroxy)propyl)-8-methoxyquinoline (118)



To a solution of the allyl compound **104** (0.4 g, 2.01 mmol) in *tert*-butanol (10 mL) and water (10 mL) was added potassium ferricyanide (III) (1.98 g, 6.0 mmol), potassium carbonate (0.829 g, 6.0 mmol) and osmium tetroxide (4% aq., 0.13 mL, 0.02 mmol). The mixture was stirred at rt for 24 h then sodium sulphite (0.756 g, 6 mmol) was added and stirred at rt for 2.5 h. The *tert*-butanol was evaporated and the resulting mixture was diluted with water (10 mL) then extracted with ethyl acetate (2 × 30 mL), the combined organic layers were eashed with brine (30 mL) dried Na_2SO_4 and evaporated to give a yellow oil (0.4 g). Purification by column chromatography on silica gel (5% methanol/dichloromethane) gave **118** as a yellow oil (0.378 g, 1.62 mmol, 81 %); ν_{max} (cm^{-1}) 3354 (OH); ^{1}H NMR (500 MHz, chloroform-*d*) δ ppm 2.98 - 3.07 (2H, m, H(11)), 3.19 (2H, br s, OH), 3.52 (1H, dd, *J*=11.4, 6.1 Hz, H(13)), 3.65 (1H, dd, *J*=11.4, 3.4 Hz, H(13)'), 4.01 - 4.08 (1H, m, H(12)), 4.10 (3H, s, OCH_3), 7.33 (1H, dd,

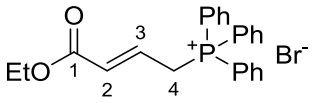
J=8.3, 4.1 Hz, H(3)), 7.39 (1H, d, *J*=8.4 Hz, H(6)), 7.47 (1H, d, *J*=8.4 Hz, H(7)), 8.08 (1H, dd, *J*=8.3, 1.5 Hz, H(4)), 8.84 (1H, dd, *J*=4.1, 1.5 Hz, H(2)); ^{13}C NMR (125 MHz, chloroform-*d*) δ ppm 34.6 (C(11)), 62.4 (OCH_3), 66.0 (C(13)), 72.6 (C(12)), 120.9 (C(6)), 123.4 (C(3)), 128.8 (C(5)), 129.9 (C(7)), 130.9 (C(8)), 136.3 (C(4)), 142.4 (C(10)), 149.6 (C(2)), 153.6 (C(9)); *m/z* (ESI^+) 234 ($[M+H]^+$, 100%); HRMS $C_{13}H_{16}NO_3^+$ requires: 234.125, found: 234.1121.

ethyl (*E*)-4-(diethoxyphosphoryl)but-2-enoate (119)[165]



Ethyl 4-bromocrotonate (80% technical) (1.43 mL, 9.12 mmol) was added to triethyl phosphite (1.56 mL, 9.12 mmol) and the mixture was stirred at 130 °C for 4 h under reflux. The resulting yellow oil was purified by column chromatography on silica gel (ethyl acetate:hexane, 4:1) to give **119** as a colourless oil (1.91 g, 7.62 mmol, 84%); ν_{max} (cm^{-1}) 1716 (C=O); ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 1.23 - 1.42 (9H, m, 3 × C*H*$_3$CH$_2$O), 2.75 (2H, ddd, *J*=22.8, 8.0, 1.5 Hz, H(4)), 4.04 - 4.26 (6H, m, CH$_3$C*H*$_2$O), 5.91 - 6.01 (1H, m, H(2)), 6.88 (1H, dq, *J*=15.4, 7.7 Hz, H(3)); ^{13}C NMR (125 MHz, chloroform-*d*) δ ppm 14.2 ($CO_2CH_2CH_3$), 16.4 (d, *J*=6.7 Hz, $POCH_2CH_3$), 30.6 (d, *J*=139.2 Hz, C(4)), 60.4 ($CO_2CH_2CH_3$), 62.2 (d, *J*=6.7 Hz, $POCH_2CH_3$), 125.8 (d, *J*=13.4 Hz, C(2)), 137.4 (d, *J*=11.5 Hz, C(3)), 165.6 (d, *J*=2.9 Hz, C=O).

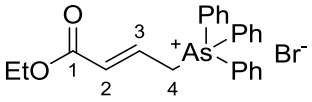
(*E*)-(4-ethoxy-4-oxobut-2-en-1-yl)triphenylphosphonium bromide (120)



To a solution of ethyl-4-bromocrotonate (80% technical grade) (1 mL, 7.26 mmol) in toluene (5 mL) was added triphenylphosphine (1.98 g, 7.55 mmol) the solution was stirred at rt for 16 h then filtered to give a white solid (2.88 g). Recrystallization from hot acetonitrile (27 mL) and ethyl acetate (36 mL) gave **120** as a white solid (1.42 g, 5.37 mmol, 74 %), mp 176-178 °C (dec) (lit.[166] 189-191 (dec)); ν_{max} (cm^{-1}) 1714 (C=O); ^{1}H NMR (500 MHz, chloroform-*d*) δ ppm 1.22 (3H, t, *J*=7.3 Hz, OCH_2CH_3), 4.11 (2H, q, *J*=7.3 Hz, OCH_2CH_3), 5.24 (2H, dd, *J*=16.4, 7.6 Hz, H(4)), 6.48 (1H, dd, *J*=15.4, 4.7 Hz, H(2)), 6.70 (1H, dtd, *J*=15.4, 7.6, 6.6 Hz, H(3)), 7.69 (6H, td, *J*=7.7, 3.5 Hz, *m*-Ph), 7.80 (3H, td, *J*=7.4, 1.6 Hz, *p*-Ph), 7.87 (6H, dd, *J*=12.6, 7.4 Hz, *o*-Ph); ^{13}C NMR (125 MHz, chloroform-*d*) δ ppm 14.1 (OCH_2CH_3), 27.6 (d, *J*=49.9

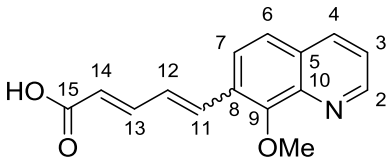
Hz, C(4)), 60.8 (O*C*H$_2$CH$_3$), 117.5 (d, *J*=86.4 Hz, *i*-Ph), 130.4 (d, *J*=12.5 Hz, *o*-Ph), 130.8 (d, *J*=13.4 Hz, C(2)), 132.2 (d, *J*=9.6 Hz, C(3)), 133.9 (d, *J*=9.6 Hz, *m*-Ph), 135.2 (d, *J*=2.9 Hz, *p*-Ph), 165.1 (d, *J*=2.9 Hz, C=O).

(*E*)-(4-ethoxy-4-oxobut-2-en-1-yl)triphenylarsonium bromide (121)[131]



To a solution of ethyl-4-bromocrotonate (80% technical grade) (2.35 g, 9.74 mmol) in toluene (1 mL) was added triphenylarsine (2.49 g, 8.12 mmol) the solution was stirred at reflux for 1.5 h then left standing at rt for 7 days (flask was left open to allow some toluene to evaporate). The white solid was filtered and washed with cold toluene (8 mL) then dried under vacuum to give **121** as a white solid (3.05 g, 6.11 mmol, 75 %), mp 141-145 °C (lit.[131] 151-152); ^{1}H NMR (500 MHz, chloroform-*d*) δ ppm 1.22 (3H, t, *J*=7.1 Hz, OCH$_2$C*H*$_3$), 4.11 (2H, q, *J*=7.1 Hz, OC*H*$_2$CH$_3$), 5.28 (2H, d, *J*=8.2 Hz, H(4)), 6.45 (1H, d, *J*=15.4 Hz, H(2)), 6.82 (1H, dt, *J*=15.4, 8.2 Hz, H(3)), 7.68 (6H, t, *J*=7.7 Hz, *m*-Ph), 7.75 (3H, t, *J*=7.7 Hz, *p*-Ph), 7.82 (6H, d, *J*=8.0 Hz, *o*-Ph); ^{13}C NMR (125 MHz, chloroform-*d*) δ ppm 14.2 (OCH$_2$*C*H$_3$), 29.9 (C(4)), 60.8 (O*C*H$_2$CH$_3$), 120.7 (*i*-Ph), 130.4 (C(2)), 131.0 (*o*-Ph), 133.2 (*m*-Ph), 133.5 (C(3)), 134.4 (*p*-Ph), 165.1 (C(1)).

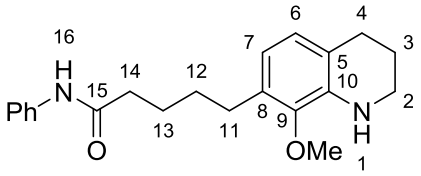
(2*E*)-5-(8-methoxyquinolin-7-yl)penta-2,4-dienoic acid (122)



To a suspension of the phosphonium bromide **120** (400 mg, 1.51 mmol) in anhydrous THF (13 mL) was added potassium *tert*-butoxide (227 mg, 2.02 mmol) under a nitrogen atmosphere, after stirring at rt for 1 h the mixture was cooled to 0 °C. To the yellow mixture was added a solution of aldehyde **107** (188 mg, 1.01 mmol) in anhydrous THF (3 mL) dropwise over 5 min, and then stirred at rt for 1 h. The reaction was partitioned between ethyl acetate (50 mL) and water (30 mL), the organic layer was washed with brine (30 mL), dried Na_2SO_4 and evaporated to give a yellow oil (539 mg). To a solution of this oil in THF (10 mL) was added lithium hydroxide (0.5 M, aq. 10 mL) and the mixture stirred at 70 °C for 15 h, Cooled to rt and then washed with diethyl ether (10 mL). The aqueous was acidified to pH 5 with 1M hydrochloric acid and then

extracted with ethyl acetate (4 × 25 mL), the combined organic layers were dried $MgSO_4$ and evaporated to give **122** (inseparable 1:1 mixture of *E*:*Z* isomers) as a yellow solid (152 mg, 0.595 mmol, 59%); ^{1}H NMR (400 MHz, methanol-d_4) δ ppm 4.01 (3H, s), 4.07 (3H, s), 6.12 (2H, d, *J*=15.2 Hz), 6.64 (1H, t, *J*=11.9 Hz), 7.15 - 7.29 (2H, m), 7.48 - 7.61 (5H, m), 7.63 - 7.77 (3H, m), 7.90 (1H, d, *J*=8.8 Hz), 8.33 (1H, dd, *J*=8.3, 1.5 Hz), 8.37 (1H, dd, *J*=8.3, 1.8 Hz), 8.83 - 8.94 (2H, m); ^{13}C NMR (125 MHz, methanol-d_4) δ ppm 62.9, 63.6, 123.1, 123.6, 124.5, 125.0, 125.5, 125.6, 129.9, 130.0, 130.1, 130.2, 130.6, 131.3, 131.7, 134.0, 135.0, 138.2, 138.4, 141.8, 143.2, 143.7, 146.5, 151.1, 151.2, 155.0, 155.1, 170.2, 170.4.

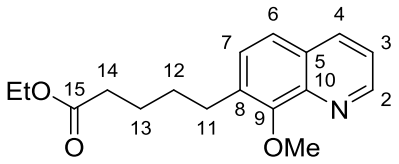
5-(8-methoxy-1,2,3,4-tetrahydroquinolin-7-yl)-*N*-phenylpentanamide (123)



To a solution of **114** (63 mg, 0.191 mmol) in dichloromethane (1 mL) and methanol (0.5 mL) was added 10% palladium on activated carbon (19 mg, 0.018 mmol). The flask was then evacuated and filled with nitrogen three times to remove oxygen, finally the flask was evacuated and then filled with hydrogen gas from a balloon. The mixture was stirred vigorously under a hydrogen balloon for 32 h. Then a further 5 mg of 10% palladium on activated carbon was added and after evacuating and back filling with nitrogen three times then evacuating and back filling with hydrogen gas from a balloon the mixture was stirred vigorously under a hydrogen balloon for another 16 h. The mixture was filtered through a thin layer of celite and washed with a 1:1 mixture of methanol and dichloromethane (5 ml) then the filtrates were evaporated. Purification by column chromatography on silica gel (hexane:ethyl acetate, 3:2) gave **123** as a white solid (54 mg, 0.160 mmol, 84%), mp 87-88 °C; ν_{max} (cm^{-1}) 3298 (NH), 1651 (C=O); ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 1.65 - 1.75 (2H, m, H(12)), 1.76 - 1.85 (2H, m, H(13)), 1.91 - 2.00 (2H, m, H(3)), 2.39 (2H, t, *J*=7.5 Hz, H(14)), 2.64 (2H, t, *J*=7.4 Hz, H(11)), 2.76 (2H, t, *J*=6.4 Hz, H(4)), 3.29 - 3.37 (2H, m, H(2)), 3.74 (3H, s, OCH_3), 4.30 (1H, br s, H(1)), 6.44 (1H, d, *J*=7.8 Hz, H(7)), 6.71 (1H, d, *J*=7.8 Hz, H(6)), 7.11 (1H, t, *J*=7.3 Hz, *p*-Ph), 7.33 (2H, t, *J*=7.9 Hz, *m*-Ph), 7.38 (1H, br s, H(16)), 7.52 (2H, d, *J*=7.9 Hz, *o*-Ph); ^{13}C NMR (125 MHz, chloroform-*d*) δ ppm 22.1 (C(3)), 25.6 (C(4)), 26.6 (C(11)), 28.9 (C(12)), 30.3 (C(13)), 37.7 (C(14)), 41.6 (C(2)), 59.6 (OCH_3), 117.0 (C(7)), 119.9 (*o*-Ph), 120.5 (C(5)), 124.2 (C(8)), 125.0 (C(6)), 129.0 (*m*-Ph), 132.0 (*p*-

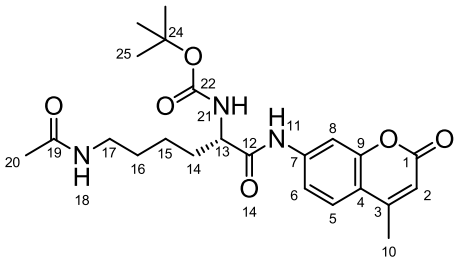
Ph), 138.1 (C(10)), 138.1 (*i*-Ph), 144.2 (C(9)), 171.5 (C(15)); *m/z* (EI^+) 338 (M^+, 100%); HRMS $C_{21}H_{26}N_2O_2^+$ requires: 338.1994, found: 338.1993.

ethyl 5-(8-methoxyquinolin-7-yl)pentanoate (125)



To a solution of diene **113** (100 mg, 0.353 mmol) in ethyl acetate (1 mL) was added 10% palladium on activated carbon (19 mg, 0.018 mmol). The flask was then evacuated and filled with nitrogen three times to remove oxygen, finally the flask was evacuated and then filled with hydrogen gas from a balloon. The mixture was stirred vigorously under a hydrogen balloon for 5 h then filtered through a thin layer of celite and washed with dichloromethane (10 ml) then the filtrates were evaporated. Purification by column chromatography on silica gel (hexane:ethyl acetate, 3:2) gave **125** (24 mg, 0.084 mmol, 24%) as a colourless oil [mixture of ethyl and methyl esters] which was not further purified before reaction with aniline.

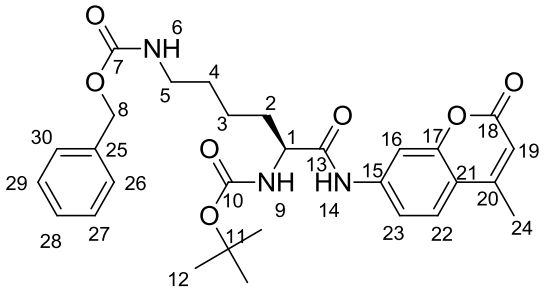
7-*tert*-butyl *N*-{5-acetamido-1-[(4-methyl-2-oxo-2*H*-chromen-7-yl)carbamoyl]pentyl}carbamate (MAL)[85]



To an oven-dried round bottomed flask was added Boc-Lys(Ac)-OH (0.3 g, 1.04 mmol, 1.0 eq), 7-amino-4-methylcoumarin (0.19 g, 1.08 mmol, 1.04 eq) and anhydrous pyridine (8 ml). The solution was cooled to -10 °C (salt-ice bath) and under an nitrogen atmosphere phosphoryl chloride (0.11 ml, 1.18 mmol, 1.13 eq) was added dropwise over 5 min. The solution was stirred at -10 °C for 30 min then quenched with ice/water (30 ml) and extracted with ethyl acetate (3 × 35 ml), some hydrochloric acid (2M aq.) was added to aid the separation. Combined organics were washed with sodium hydrogen carbonate (sat. aq., 2 × 20 ml) and brine (20 ml) then dried $MgSO_4$ and concentrated. Purification by column chromatography on silica gel (5% methanol/EtOAC) gave MAL as a beige solid (0.171 g, 0.384 mmol, 37%), mp 101-102

°C (lit. 175 °C)[85]; $[\alpha]_D^{20}$ +3.2 (*c* 0.5, methanol); ν_{max} (cm^{-1}) 3302 (NH), 1730 (C=O), 1676 (C=O); ^{1}H NMR (600 MHz, methanol-d_4) δ ppm 1.34 - 1.61 (13H, m, $C(CH_3)_3$, $C(16)H_2$ and $C(15)H_2$), 1.66 - 1.76 (1H, m, C(14)H), 1.76 - 1.86 (1H, m, C(14)H`), 1.91 (3H, s, $C(10)H_3$), 2.43 (3H, s, $C(20)H_3$), 3.13 - 3.21 (2H, m, $C(17)H_2$), 4.18 (1H, dd, *J*=8.7, 5.3 Hz, C(13)H), 6.19 (1H, s, C(2)H), 7.40 (1H, dd, *J*=8.7, 1.5 Hz, C(6)H), 7.64 (1H, d, *J*=8.7 Hz, C(5)H), 7.77 (1H, d, *J*=1.1 Hz, C(8)H); ^{13}C NMR (150 MHz, methanol-d_4) δ ppm 18.6 (C(10)), 22.6 (C(20)), 24.4 (C(15)), 28.8 (C(25)), 30.1 (C(16)), 33.0 (C(14)), 40.2 (C(17)), 56.9 (C(13)), 80.8 (C(24)), 107.9 (C(2)), 113.6 (C(8)), 117.1 (C(6)), 117.1 (C(4)), 126.6 (C(5)), 143.4 (C(7)), 155.2 (C(3 and 9)), 158.1 (C(22)), 163.2 (C(1)), 173.2 (C(19)), 174.2 (C(12)); *m/z* (ES^+) 468 ($[M+Na]^+$, 100%); HRMS $C_{23}H_{31}N_3O_6Na^+$ requires: 468.2111, found: 468.2090.

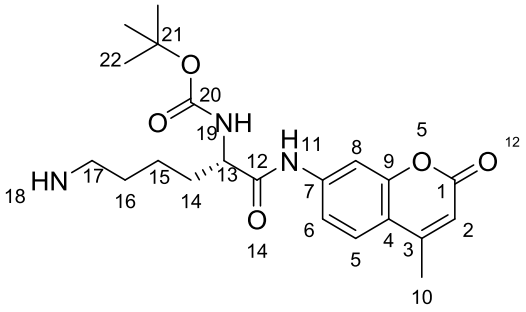
benzyl *N*-(5-{[(*tert*-butoxy)carbonyl]amino}-5-[(4-methyl-2-oxo-2*H*-chromen-7-yl)carbamoyl]pentyl)carbamate (129)



To a solution of *N*(α)-Boc-*N*(ε)-Cbz-(L)-lysine (0.380 g, 1.0 mmol, 1.0 eq) and 7-amino-4-methylcoumarin (0.175 g, 1.0 mmol, 1.0 eq) in anhydrous pyridine (8 ml) at -10 °C was added phosphoryl chloride (0.14 ml, 1.5 mmol, 1.5 eq) dropwise over 2 min. The solution was then stirred at -10 °C for 1 h, then poured onto 30 ml ice/water and extracted with ethyl acetate (40 ml). The organic was washed with hydrochloric acid (0.25 M, aq., 35 ml), water (30 ml), brine (20 ml) then dried $MgSO_4$, and evaporated. Purification by column chromatography on silica gel (dichloromethane to 20% ethyl acetate/dichloromethane) gave **129** as a white foamy solid (0.196 g, 0.365 mmol, 37%), mp 78-81 °C; ν_{max} (cm^{-1}) 3315 (NH), 1684 (C=O); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 1.21 - 1.47 (11H, m, $C(CH_3)_3$ and $C(3)H_2$), 1.52 - 1.70 (2H, m, $C(2)H_2$), 2.38 (3H, s, $C(24)H_3$), 2.92 - 3.03 (2H, m, $C(4)H_2$), 3.31 - 3.35 (2H, m, $C(5)H_2$), 3.98 - 4.08 (1H, m, H(1)), 4.97 (2H, s, $C(8)H_2$), 6.24 (1H, s, H(19)), 7.10 (1H, d, *J*=7.3 Hz, H(9)), 7.23 (1H, t, *J*=5.0 Hz, H(6)), 7.25 - 7.39 (5H, m, Ph), 7.47 (1H, d, *J*=8.7 Hz, H(23)), 7.70 (1H, d, *J*=8.7 Hz, H(22)), 7.76 (1H, s, H(16)), 10.39 (1H, s, br., H(6)); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 18.0 (C(24)), 22.9 (C(3)), 28.2 (C(12)), 29.1 (C(4)), 31.2

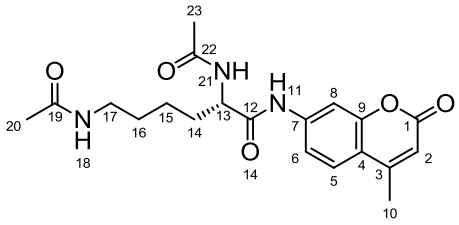
(C(2)), 39.9 (C(5)), 55.3 (C(1)), 65.1 (C(8)), 78.1 (C(11)), 105.6 (C(16)), 112.2 (C(19)), 115.0 (C(21)), 115.2 (C(23)), 125.9 (C(22)), 127.7 (C(26 and 33)), 127.7 (C(28)), 128.3 (C(27 and 29)), 137.2 (C(25)), 142.3 (C(15)), 153.1 (C(20)), 153.6 (C(17)), 155.6 (C(10)), 156.1 (C(7)), 160.0 (C(18)), 172.3 (C(13)); *m/z* (ES^-) 536 (M^-, 100%); HRMS $C_{29}H_{34}N_3O_7^-$ requires: 536.2397, found: 536.2397.

tert-butyl _N_-[(1_S_)-5-amino-1-[(4-methyl-2-oxo-2_H_-chromen-7-yl)carbamoyl]pentyl]carbamate (130)



To a solution of **129** (170 mg, 0.316 mmol) in methanol (5 ml) was added 10% activated palladium on activated carbon. The flask was then evacuated and filled with nitrogen three times to remove oxygen, finally the flask was evacuated and then filled with hydrogen gas from a balloon. The mixture was stirred vigorously under a hydrogen balloon for 5 h, then filtered through a thin layer of celite and washed with methanol (10 ml) the combined filtrates were evaporated. Purification by column chromatography on silica gel (1% ammonia (30% aq.), 14% methanol, 85% ethyl acetate to 5% ammonia (30% aq.), 15% methanol, 80% ethyl acetate) gave **130** as a cream solid (53 mg, 0.131 mmol, 42%), mp 98-99 °C; $[\alpha]_D^{20}$ -9.1 (*c* 0.5, methanol); ; ν_{max} (cm^{-1}) 3299 (NH), 1684 (broad, 3 × C=O); ^{1}H NMR (500 MHz, chloroform-*d*) δ ppm 1.28 - 1.61 (13H, m, $C(CH_3)_3$, $C(16)H_2$ and $C(15)H_2$), 1.63 - 1.79 (1H, m, H(14)), 1.80 - 1.94 (1H, m, H(14)`), 2.35 (3H, s, $C(10)H_3$), 2.62 (2H, br s, $C(17)H_2$), 2.75 (2H, br s, NH_2), 4.35 (1H, br s, H(13)), 5.55 (1H, br s, H(19)), 6.10 (1H, br s, H(2)), 7.22 - 7.29 (1H, m, H(6)), 7.40 (1H, d, *J*=8.0 Hz, H(5)), 7.66 (1H, s, H(8)), 9.73 (1H, br s, H(11)); ^{13}C NMR (125 MHz, chloroform-*d*) δ ppm 18.6 (C(10)), 22.9 (C(15)), 28.4 (C(22)), 32.0 (C(16)), 32.2 (C(14)), 41.3 (C(17)), 55.3 (C(13)), 80.6 (C(21)), 107.2 (C(2)), 113.2 (C(8)), 115.6 (C(4)), 115.8 (C(6)), 125.0 (C(5)), 141.6 (C(7)), 152.6 (C(9)), 154.0 (C(3)), 156.6 (C(20)), 161.2 (C(1)), 171.7 (C(12)); *m/z* (ES^+) 404 ($[M+H]^+$, 98%); HRMS $C_{21}H_{30}N_3O_5^+$ requires: 404.2185, found: 404.2180.

(*2S*)-2,6-diacetamido-*N*-(4-methyl-2-oxo-2*H*-chromen-7-yl)hexanamide (131)



To a solution of MAL (0.178 g, 0.40 mmol) in dichloromethane (3 mL) was added 2M HCl in diethyl ether (3 mL), a precipitate formed and the suspension was stirred at rt for 3 h. A further 3 mL of 2M HCl in diethyl ether was added and then stirred at rt for 2.5 h. The solvent was evaporated and the residue partitioned between sodium hydrogen carbonate (sat. aq, 40 mL) and ethyl acetate (50 mL), and the aqueous layer was extracted with ethyl acetate (2 × 25 mL). The combined organics were washed with brine (20 mL), dried Na_2SO_4 and concentrated to give a beige solid (20 mg). The aqueous layer was also evaporated to give a white solid which included some inorganic salts (0.525 g).

To the concentrated aqueous layer suspended in acetonitrile (4 mL) and *N*,*N*-diisopropylethylamine (0.15 mL, 0.88 mmol, 2.2 eq) at -10 °C under nitrogen was added acetic anhydride (0.083 mL, 0.88 mmol, 2.2 eq) slowly over 5 min. Stirred for 2 h, warming to 0 °C during that time. The reaction was quenched with water (30 mL) and then extracted with ethyl acetate (100 mL and 2 × 40 mL) the combined organics were washed with brine (30 mL) dried $MgSO_4$ and concentrated to give a white solid (95 mg). The aqueous layer was extracted with 20% ethanol/chloroform (2 × 40 mL), these organics were washed with brine (20 mL) and dried $MgSO_4$ to give more white solid (30 mg). TLC showed that both solids contained product, and so they were combined and then purified by column chromatography (8% to 10% methanol/ethyl acetate) to give **131** as a white solid (91 mg, 0.235 mmol, 59%), mp 243-245 °C; $[\alpha]_D^{20}$ -7.6 (*c* 0.5, methanol); ν_{max} (cm^{-1}) 3304 (NH), 1717 (C=O), 1693 (C=O), 1679 (C=O), 1644 (C=O); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 1.20 - 1.45 (4H, m, H(15) and H(16)), 1.54 - 1.72 (2H, m, H(14)), 1.76 (3H, s, H(20)), 1.88 (3H, s, H(23)), 2.39 (3H, s, H(10)), 3.00 (2H, app. q, *J*=5.8 Hz, H(17)), 4.31 - 4.40 (1H, m, H(13)), 6.26 (1H, s, H(2)), 7.49 (1H, d, *J*=8.7 Hz, H(8)), 7.71 (1H, d, *J*=8.7 Hz, H(9)), 7.75 - 7.86 (2H, m, H(6) and H(18)), 8.20 (1H, d, *J*=7.4 Hz, H(21)), 10.49 (1H, s, H(11)); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 18.0 (C(10)), 22.4 (C(15)), 22.6 (C(20)), 23.0 (C(23)), 28.9 (C(16)), 31.5 (C(14)), 38.3 (C(17)), 53.6 (C(13)), 105.7 (C(6)), 112.3 (C(2)), 115.0 (C(4)), 115.3 (C(8)), 125.9 (C(9)), 142.2 (C(7)), 153.1 (C(3)), 153.6 (C(5)), 160.0 (C(1)), 169.0 (C(19)), 169.5

(C(22)), 171.8 (C(12)); *m/z* (CI^+) 388 ($[M+H]^+$, 44%), 370 ($[M+H\text{-water}]^+$, 82%); HRMS $C_{20}H_{25}N_3O_5^+$ requires: 388.1873, found: 388.1862.

7.2 *In vitro* HDAC inhibition assays

Recombinant HDAC isoforms were used. HDAC2 (1−488, His-tag), HDAC3-NCoR1, HDAC6 (His-tag), Fluor-de-Lys SIRT1, Fluor-de-Lys developer II, and TSA were purchased from Enzo Life Sciences, and porcine pancreatic trypsin (type IX-S) was from Sigma. HDAC1 (C-Flag), HDAC2 (C-His-tag) and HDAC3-NCoR2 were purchased from Tebu Bio. HDAC8 was expressed in Escherichia coli BL21 cells and purified with a His-tag, which was removed prior to final purification by size exclusion chromatography.

Endpoint assay

The in vitro HDAC assay used was based on a homogeneous fluorogenic HDAC assay.[88] Inhibitor solutions were prepared by serial dilution of a 5 mM DMSO stock solution with assay buffer except for the fragments when screening at 1 mM, these were prepared by diluting a 25 mM DMSO stock solution with assay buffer. Assays were carried out in a 96-well white non-binding surface microplate, to 10 μL of inhibitor solution was added purified recombinant HDAC enzyme (HDAC1, 120 ng; HDAC2, 85 ng; HDAC3-NCoR1, 25 ng; HDAC6, 280 ng; HDAC8, 400 ng) in 20 μL of assay buffer comprising 50 mM Tris/Cl, pH 8.0, 137 mM NaCl, 2.7 mM KCl, 1 mM MgCl2, 1 mg/mL BSA (for HDAC8 assays the buffer also contained 10% v/v PEG 8000. After incubation at 20−23 °C for the appropriate time (HDAC1, 1 h; HDAC2, HDAC3, and HDAC6, 3 h; HDAC8, 15 min) the fluorescent substrate (for HDAC1, HDAC2, HDAC3-NCoR1, and HDAC8, MAL (20 μL), to give concentrations of 60, 80, 14, and 200 μM; and for HDAC6, Fluor-de-Lys SIRT1, to give a concentration of 13 μM) was added and the plate was incubated for 30-60 min at 37 °C. A developer solution (for HDAC1, HDAC2, HDAC3-NCoR1, and HDAC8, 50 μL of 10 mg/mL trypsin and 2 μM TSA in assay buffer; for HDAC6 Fluor-de-Lys developer II and 2 mM TSA in 50 mMTris/Cl, pH 8.0, 137 mMNaCl, 2.7 mMKCl, 1 mMMgCl2) was then added and the plate incubated for 30 min at rt before the fluorescence was measured on a BMG FLUOstar Optima plate reader with excitation at 380 nm and emission at 460 nm. A well which contained 20 μL buffer instead of the enzyme solution was used as a zero deacetylation control and the fluorescence value of this well was subtracted from all other wells. Some wells contained 10 μL buffer instead of an inhibitor solution to be the

maximum deacetylation control and the average fluorescence value of these wells was used to calculate a percentage inhibition for the other wells. OriginPro 8 was used to determine IC50 values from the sigmoidal line fitted to a graph of log[concentration] against the average percentage inhibition from two independent experiments, with at least six different concentrations. The SEM is the calculated standard error in the IC50 value of the fitted line.

'Progression method' assay

Inhibitor solutions were prepared by serial dilution of a 5 mM DMSO stock solution with assay buffer. In a 96 well white non-binding surface™ microplate fluorescent substrate Ac-Lys(Ac)-AMC (10 µL, 250 µM in assay buffer), inhibitor solution (10 µL) and Lys-C peptidase enzyme (10 µL, 0.2 U/mL in assay buffer) were added to each well. To a control well, assay buffer (10 µL) was added instead of inhibitor solution. Following a 10 min equilibration of both the plate and the enzyme solution to assay temperature (30 °C) HDAC3-NCoR1 (20 µL, 5 ng/µL) was added at the same time to 8 wells using a multichannel pipette. The plate was then incubated at 30 °C and the fluorescence of each well measured every minute for 1 h, with excitation at 380 nm and emission at 460 nm.

The data obtained were fitted to the integrated rate equation for slow binding inhibitors [$F = v_s t + (v_0 - v_s)(1 - \exp(-k_{obs}t))/k_{obs}$] using OriginPro 8.6. The calculated k_{obs} values were then plotted against inhibitor concentration to determine the mechanism of inhibition (see Gottesfeld *et al.* for details).[67]

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